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PENETRATION AND ACCUMULATION OF PETROLEUM SPRAY OILS IN THE LEAVES, TWIGS, AND FRUIT OF CITRUS TREES[1]

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(WITH FOURTEEN FIGURES)

Introduction

The chief objection to the use of petroleum oils on citrus trees is the injury which may result. Various writers have noted some of the manifestations of injury, such as leaf and fruit drop, off-bloom, retardation of bloom, reduction in fruit set, failure of fruit to color properly, poor flavor, thickness of rind, softening of rind, susceptibility to breakdown and decay, and killing of twigs. Under certain conditions little understood, these injuries are rather extensive and are of considerable economic importance.

Many growers have feared that an accumulation of oil in the trees might take place from year to year, and that after a period of 10 to 15 years sufficient oil might accumulate to cause permanent injury to the health of the trees and to result in a reduction of quantity or quality of fruit.

In spite of these adverse effects, emulsions of highly refined petroleum oils are recognized as being among the best insecticides for the control of various insect pests of citrus, such as red scale, *Aonidiella aurantii* (Maskell); purple scale, *Lepidosaphes beckii* (Newman); black scale, *Saissetia oleae* (Bernard); citricola scale, *Coccus pseudomagnoliarum* (Kuwana); and red spider, *Paratetranychus citri* (McGregor).

The importance of oil spray as an insecticide is seen in the fact that during the year ending June 30, 1930, the growers in the county of Los Angeles alone spent $1,363,000 for scale insect control by means of oil sprays and fumigation (**12**). The average cost over the previous seven

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years was $1,377,000 and the average number of acres treated per year was 41,524. The acreage treated with oil has gradually been increased until during the year 1930, 55 per cent. of the total treated acreage was treated by means of oil spray. Approximately 94,000 acres of citrus orchards in California were sprayed with oil emulsions during 1931 (**2**).

KNIGHT, CHAMBERLIN, and SAMUELS (**11**) have investigated the penetration and location of spray oils in citrus trees, but their work on penetration was limited to the use of undiluted oils. A study of the penetration and accumulation of oil applied as emulsions seems very desirable, as this is the form in which the oil is used in the commercial orchard.

The present paper contains data relating to (1) the entrance of the oil into the leaves and twigs, (2) the movement of the oil in the tree, (3) the tissues in which the oil is finally deposited, (4) the rate of disappearance of oil from the tissues, and (5) the anatomical effects which result from the use of spray oils.

Histological studies

OILS USED AND THEIR SPECIFICATIONS

The spray oils and oil emulsions used in most of these experiments are materials commonly used in the commercial spraying of citrus trees in California. The specifications of the oils are given in table I.

TABLE I

SPECIFICATIONS OF OILS USED IN THESE EXPERIMENTS

OILS	VISCOSITY	UNSULPHONATABLE RESIDUE
		%
Oronite technical	100–105	99
Oronite X (a special oil)	100	85
Oronite cosmetic	70	99
Oronite mineral seal	50	94
Special 80	80	85
Volck concentrate	100–105	99
Volck light	50	94
Avon heavy	95	94

The "tank-mix" emulsions referred to in this paper are emulsions made by placing the emulsifier in the spray tank with the water. When the water and emulsifier are mixed and the latter is dissolved, the undiluted oil is added. The spray machine mixes these until an emulsion is formed.

In these experiments the percentage of oil used refers to the percentage of actual oil in the completed emulsion; that is, when Volck Concentrate (a

commercially prepared emulsion) is used, enough more of the heavy prepared emulsion is added to make up for the 18 per cent. of water already in the heavy emulsion.

METHODS

Two methods of investigation have been followed in these experiments. One method consists of a histological study of the penetration, location, and accumulation of the oil in the tissues of the plant. The other method consists of an extraction and quantitative measurement of the oil found in a given quantity of plant tissue.

TECHNIQUE

There have heretofore been no staining methods which satisfactorily distinguish between the natural plant oils and the petroleum spray oils in the plant. It is difficult for anyone using the methods of KNIGHT, CHAMBERLIN, and SAMUELS (**11**) to be certain which oils are plant oils and which are spray oils, since they both stain red. This is especially true of sections of the stems.

The staining methods described below have proved very useful for distinguishing between the natural oil and the petroleum spray oil in sections of citrus tissue.

The technique found most practical for the histological studies consists of cutting sections of fresh tissue from the part of the plant to be investigated. This is best done with a sliding microtome having a freezing attachment. The sections are then placed in a normal solution of potassium hydroxide or Bouin's killing solution for 20 minutes, or they may be placed directly in a dye solution made up as follows: To a saturated aqueous solution of nile blue sulphate, 0.5 per cent. of sulphuric acid is added and the mixture is boiled under a reflux condenser for 4 or 5 hours. (This solution should be made as nearly alkaline as possible without a change of color; if too much hydroxide is added the solution turns a bright red color.) A solution of 50 per cent. alcohol and 50 per cent. acetone is then saturated with oil red O dye. One part of the prepared nile blue sulphate solution is then added to 2 parts of the oil red O solution. This is allowed to settle overnight and is then filtered, after which it is ready for use.

The nile blue sulphate solution is SMITH and MAIR'S (**16**) stain for fat. It is supposed to stain fatty acids blue and to stain the neutral esters red. Most of the natural oils of citrus stain blue with this dye, but the petroleum oil stains very little or not at all. The combination of the two dyes as just described, however, gives a brown or black color to the plastids and most of the natural oils. The petroleum spray oil, the oil in the oil glands, and frequently a small quantity of plant oil or resin which lies between

the cells of a narrow band in the phloem region of twigs, give a bright red color with this dye mixture unless the sections have been previously placed in normal potassium hydroxide solution or Bouin's killing solution. If previously placed in potassium hydroxide or Bouin's solution, only the petroleum oil shows the red color. Leaf sections stain satisfactorily in one hour while stem sections require several hours, but both are better and more permanent if stained overnight. Best results have been obtained without destaining. If the dye becomes too concentrated, owing to evaporation of alcohol and acetone, large round globules of dye appear on the sections. This may be avoided by replenishing the alcohol and acetone. When stained sufficiently, the sections are rinsed in water and then mounted in glycerin jelly.

The oil red O dissolved in pyridine as recommended by PROESCHER (**15**) may also be used either with or without the nile blue sulphate. Sections stained with oil red O in pyridine show the oil more in the form of large globules than as a film between the cells of the tissue, as shown in the sections stained with the acetone-alcohol solution. The use of alcohol and acetone has a more dehydrating effect on the tissues than has the use of pyridine.

It was found desirable to avoid all rinsings and washings of the sections which were not absolutely necessary, since the oil is mostly in the form of a film between the cells of the plant tissues and the more the sections are washed or the longer they are left in any solution, the more tendency there is for the oil to become globular and to come out of the tissues into the liquid. This is especially true when the sections are in water solutions. If sections of freshly sprayed tissues are cut and placed in water for a few hours, small droplets of oil will emerge and may be found on the surface of the water. The dyes, when used according to this method, have a tendency to fade slowly and to diffuse out into the mounting medium, so that after a week or two the sharper contrasts are lost. It is possible that some other mounting medium might prove more satisfactory in this respect. Glycerin jelly is the only medium which has been tried.

Another method of staining which has proved to be of interest and which may be used to some extent in this type of work is the nascent indophenol blue method as used by DUFRENOY (**6**). This method of staining is as follows: Dissolve 1.5 gm. of thymol crystals in 100 cc. of warm water on a water bath. Add 0.5 cc. of 33 per cent. potassium hydroxide. (This solution will keep for several weeks.) Next dissolve about 0.5 gm. of p-aminodimethylanilinehydrochloride in 50 cc. of water. This makes a very light rose-colored solution. This is decolorized by adding, drop by drop, a solution of potassium hydroxide until the rose color disappears. Avoid adding too much potassium hydroxide. Add 50 cc. of the thymol

solution. Place sections of material to be stained in this solution. After a few minutes the blue, lipoid-soluble, nascent-indophenol blue appears in the solution.

This dye stains the natural oils, lipoids, and oleo resins a bright blue and the petroleum oil a reddish blue or violet. It can be used only for temporary mounts, as it lasts only about half an hour. The larger globules of petroleum oil are readily distinguished by this method, but since the oil does not stain deeply, it is often impossible to distinguish between the films of oil and the air in the intercellular spaces. This dye has the advantage of being in a water medium and there is therefore no dehydration of the section. It probably allows one to view the larger oil globules more nearly as they are in the unsectioned leaf than is possible in sections mounted in a dehydrating medium. Figure 3*C* is a section of the upper side of an orange leaf stained by this method, showing oil between the palisade cells.

Natural oils in the plant

The first histological observations were made on sections of tissues from healthy, unsprayed citrus trees of different ages. This was done to determine whether natural plant oils were present, and, if present, to determine their location and prevalence. Many small droplets of natural oil were found in the cells of the cortex, in the medullary ray cells of the stem, and in the chlorophyll-bearing cells of the mature leaves. Figure 1, *A* and *B*,

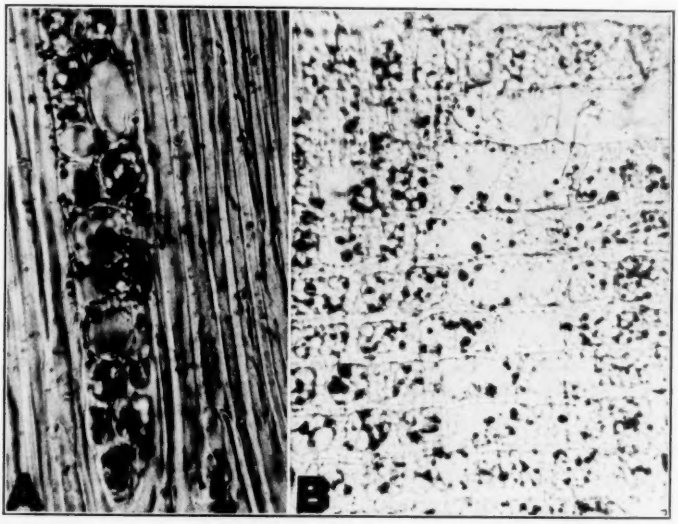

Fig. 1. Natural oil in cells of medullary ray: *A*, tangential section of unsprayed orange twig; *B*, radial section of unsprayed orange twig.

shows the natural oil in the medullary ray cells. When the leaves are very young and immature, the oil is either lacking or it is to be found only in small quantities. There is a wide variation in the quantity of oil present in different trees and in different parts of the same tree.

Entrance and location of spray oils in the plant

Leaves.—The upper surface of mature orange leaves was painted with undiluted Oronite Technical oil. The leaves were kept for the next 24 hours at a temperature ranging between 75° and 85° F. An examination at the end of this time showed that only occasionally a small amount of oil had penetrated through the leaf to the lower epidermis. The upper surface of a citrus leaf has no stomata. At a temperature of 95° F. or above, the oil sometimes passed through to the lower surface of the leaf at the end of the 24-hour period. The cuticle was rather slowly penetrated by the oil which then passed between the cells of the epidermis and the palisade layers into the spongy parenchyma (fig. 2, *A* and *B*). The final depth to which

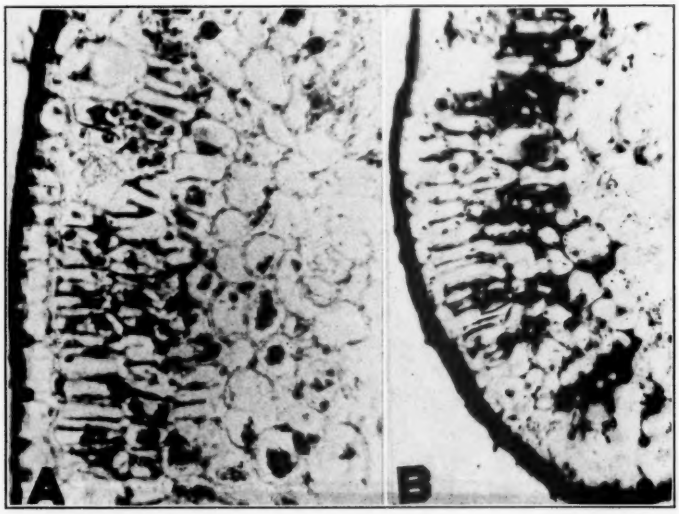

Fig. 2. Cross-section of orange leaf showing spray oil between cells of palisade region: *A*, near margin; *B*, at margin.

it penetrates depends not only on temperature but also on the quantity of oil applied. The movement of the oil appears to be due entirely to capillarity.

Oil painted on the lower surface of leaves showed a quicker penetration.

The indications were that the oil entered largely through the stomata. At first it spread in fan-like areas from the stomata (fig. 3 *A*). It may also penetrate the cuticle to some extent.

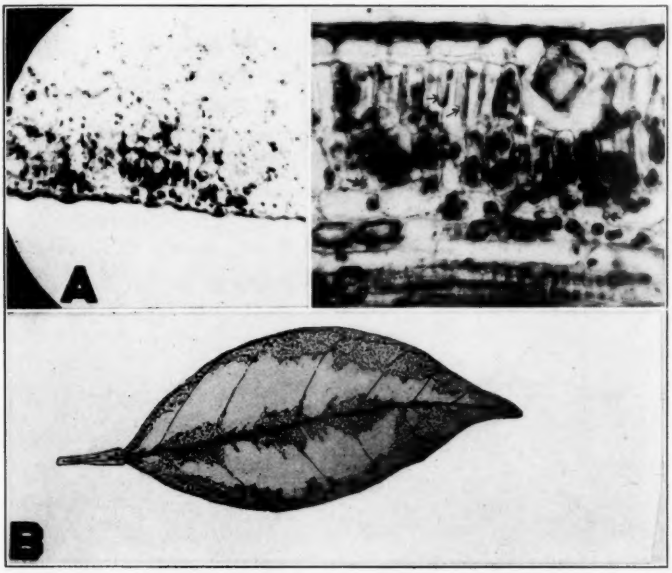

FIG. 3. *A*, section of lower surface of orange leaf showing spray oil which has entered leaf through stomata; *B*, orange leaf showing oil-soaked areas caused by oil-spray emulsion; *C*, section of leaf showing spray oil between palisade cells stained with indophenol blue. (Note rounded globules as compared with those of figure 2, *A* and *B*.) Leaf sprayed with 2 per cent. Avon Heavy.

Sections of leaves previously sprayed with oil and water emulsions showed that the oil in this form penetrated in a manner similar to that of undiluted oil, the only visible difference being in the quantity of the oil that penetrated.

When only a small quantity of oil was applied, or when an excessive amount of spreader was used, there was much less penetration of the upper epidermis and often only a small amount of oil penetrated the lower surface of the leaf. Sections of leaves which were sprayed with 2 per cent. Oronite Technical oil, using eight times the normal quantity of spreader in a tank-mix emulsion, showed a light penetration of oil which was well distributed on the lower surface of the leaves. There were only small oil-soaked areas on the margins of these leaves, and sections showed only a small quantity of oil at the margins and but little oil at any other place in the leaf.

These results indicated that under some conditions the oil may scarcely penetrate the upper surface at all. Under the conditions of ordinary commercial spray practice the oil usually concentrates along the veins, midrib, and especially the leaf margin, and there penetrates, producing an oil-soaked appearance. Figure 3 *B* is a drawing of a leaf showing an oil-soaked area, which is due to the presence of oil between the palisade cells. This oil-soaked area, when resulting from the application of the heavier oils, can be detected throughout the life of the leaf, which is sometimes more than two years. It is, however, often necessary to allow the leaf to wilt slightly before the darkened area becomes evident. The extent of the oil-soaked areas of the leaves may be used at least to some extent as an indicator of the quantity of oil in the leaves.

TWIGS.—In a preliminary test, about 8 cm. of an orange twig 1.5 cm. in diameter was painted with Oronite Technical oil which had been saturated with oil red O. One week later the twig was cut off and sectioned. The sections showed that the oil had penetrated to the pith and had spread

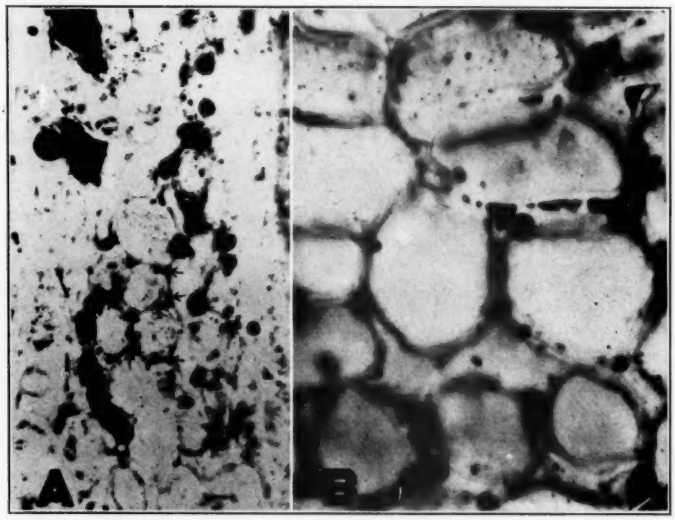

FIG. 4. Spray oil between cells of cortex of orange twig: *A*, longitudinal section; *B*, cross-section.

on the surface about 2 cm. in each direction beyond the place of application. Longitudinal sections of the stem showed that the oil had penetrated lengthwise of the stem on the inside to about the same extent that it had spread on the surface.

Other stems of various sizes were painted with dyed Oronite Technical and also with Mineral Seal oil with similar results. Twigs which had smooth bark absorbed the oil more slowly than those with split, rough bark. Cross-sections of twigs with split, rough bark showed streaks of red beneath these splits to, or nearly to, the centers of the twigs.

Sections of twigs sprayed with ordinary emulsions of Oronite Technical or Mineral Seal oil showed the same type of penetration, but to a much less extent. When the emulsions were used, the oil seldom penetrated to the cambium, although it could be found between the cells of the outer one-third of the cortex. Figure 4, *A* and *B*, shows the presence of spray oil between the cells of the cortex. Figure 5 *A* shows a section through the cortex of a normal unsprayed twig with cambium at one side and epidermis

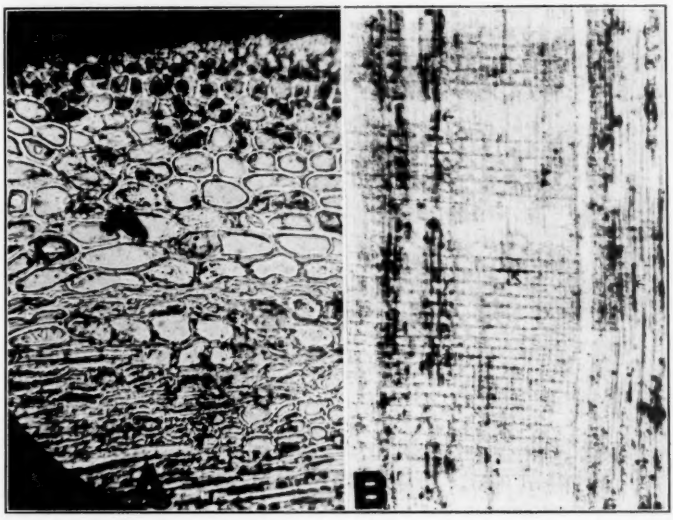

FIG. 5. *A*, longitudinal section of orange twig showing structure of cortex; *B*, radial section of orange twig showing spray oil between cells of medullary ray and in wood fibers underneath ray.

at the other. Under ordinary commercial conditions the oil would be limited to the outer one-third of this section.

Lemon, grapefruit, and orange trees were sprayed in September, 1928, with undiluted oils of various viscosities and of various percentages of sulphonatable or unsaturated hydrocarbons. Two years later, observations were made on this material. Oils of viscosity below 70 were very seldom found in these tissues and then in only very small amounts. Twigs of the trees sprayed with oil above 70 seconds' viscosity showed a distinct ring of

oil present in the lumen of the wood cells at the place where the cambium was at the time the spray was applied (fig. 6 *A*). There were also a few scattered deposits of oil deeper in the twigs. These decreased in size and frequency toward the center of the twigs although sometimes oil could be found just outside the pith of the smaller twigs in connection with gum

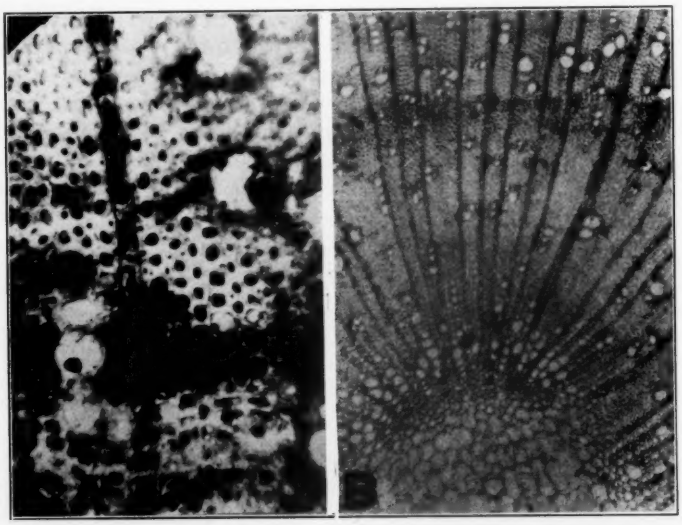

FIG. 6. Cross-section of lemon twig 2 years after spraying with pure oil, showing heavy band of oil in cells of woody cylinder: *A*, highly magnified; *B*, less highly magnified, also showing oil near pith.

deposits (fig. 6 *B*). No oil could be found outside the place where the cambium was at the time of the spray application. No such condition has been found following the application of commercial emulsions as usually used for insect control.

The quantity of spray oil which remains in a twig depends largely upon its volatility.

When undiluted oils or excessive quantities of oil emulsions are put on citrus twigs, the path of the oil which enters the twigs appears to be largely through splits if there are any in the outer bark. It then passes between the cells of the epidermis or lenticels and between the cells of the cortex until it gets to the medullary rays. It follows along the sides of the ray cells (fig. 7, *A* and *B*) past the cambium and into the woody cylinder. There it often enters and follows the lumen of the wood fibers or lubriform cells (fig. 8 *A*) lengthwise for some distance, often filling many of these

cells with the oil as it goes. Figure 8 *B* shows a section of a small twig containing spray oil between the cells just at the edge of the pith.

In contrast to this, sections of twigs sprayed with various commercial emulsions containing 2 per cent. oil or less show that the oil did not ordinarily penetrate to the cambium. Some sections of lemon twigs which had been sprayed with 3 per cent. Oronite Technical Mixol showed that a trace of oil had gone into the wood fibers. Sections of orange twigs which had been sprayed with 4 per cent. oil of 100 seconds' viscosity put on as a tank-mix emulsion also showed some oil in the wood.

FRUIT.—Sections were made of oranges from both navel and Valencia trees which had been sprayed two months previously with 2 per cent.

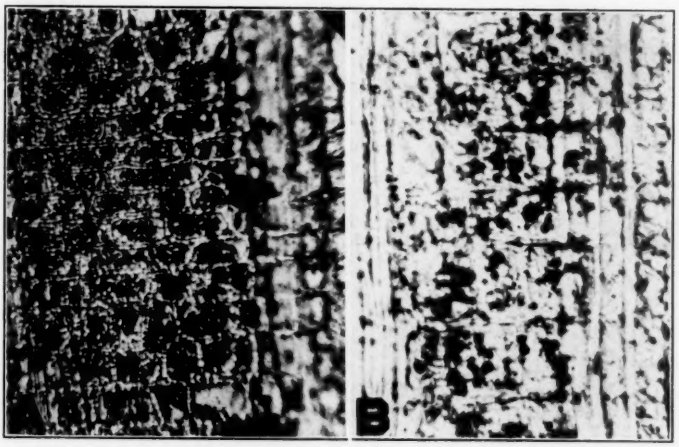

FIG. 7. Radial section of orange twig 48 hours after painting with Oronite Technical oil, showing spray oil between cells of medullary ray: *A*, faint lines of oil between cells of older portion of ray; *B*, oil between cells of new portion of ray.

tank-mix emulsions of Oronite Technical and Mineral Seal oils, and 2 per cent. emulsions of Volck Concentrate and Volck Light oils. Sections of the fruit where the Oronite Technical and the Volck Concentrate oil were used showed some oil penetration. These oils were found only in the outer 1 mm. of the rind. There are numerous oil glands in the outer portion of the rind, and the spray oil was found most abundantly between the cells which lie between these oil glands. The oil was not found evenly over the fruit but occurred only in spots and sometimes on only one side of a fruit. Sections of orange sprayed with the lighter oils, Mineral Seal and Volck Light, showed almost no spray oil present in the fruit. It was only occasionally that one could find a trace of oil.

Sections of lemons sprayed with Volck Concentrate and with Volck Light oil one month previously showed only a small amount of penetration

around the buttons of those fruits sprayed with Volck Concentrate, and no evidence of oil penetration in those sprayed with Volck Light oil.

Figure 9 *A* shows spray oil between the cells of orange rind. Figure 9 *B* is a section of lemon rind showing spray oil between the cells after the lemon had come from the packing house. This fruit showed considerable browning of the albedo on one side but the oil was also prevalent on the opposite side of the fruit.

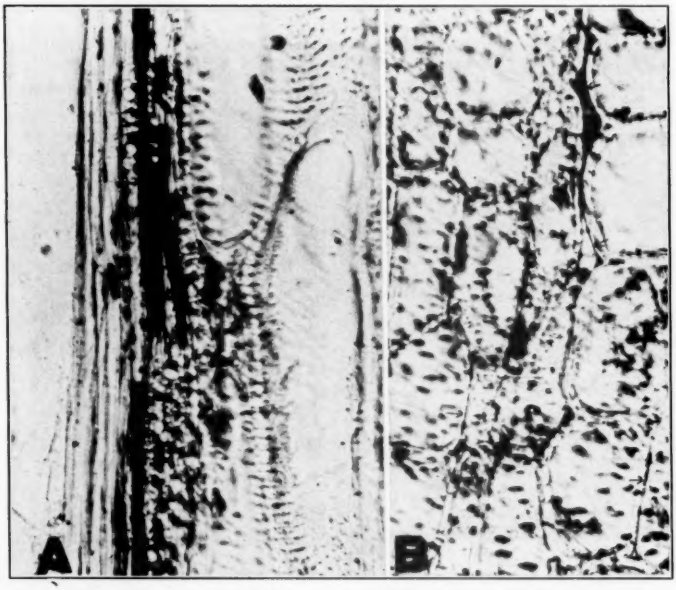

FIG. 8. Longitudinal section of orange twig 2 years after spraying with 4 per cent. emulsion of Volck Concentrate: *A*, oil in wood fibers near large xylem tubes; *B*, spray oil between cells near pith of small stem.

ROOTS.—Sections of the roots of trees which had been sprayed with undiluted oils or with emulsions have not shown any evidence of oil in them except in the case of some small trees in pots which were sprayed with undiluted oil, and the oil had run down the stem. In these plants oil was found in sections of roots taken from about 2 inches below the surface of the soil.

MOVEMENT OF SPRAY OIL IN CONDUCTING SYSTEM OF PLANT

KNIGHT, CHAMBERLIN, and SAMUELS (11) referred to the conduction of oil by the plant. Some investigators of petroleum spray oils have taken

for granted that since the oil penetrated the leaf, it must be conducted away from it into the plant stems.

To find how far the oil was conducted, twigs bearing twenty to thirty mature leaves were selected on large healthy orange trees. The twigs were used in four groups of three each. Using 2 per cent. emulsions, one group was sprayed with Volck Concentrate, another with Volck Light, a third with Oronite Technical, and a fourth with undiluted Mineral Seal oil. One twig from each group was sectioned after 24 hours, another after 3 days, and the other after 10 days, to see how far the oil had gone down the stem.

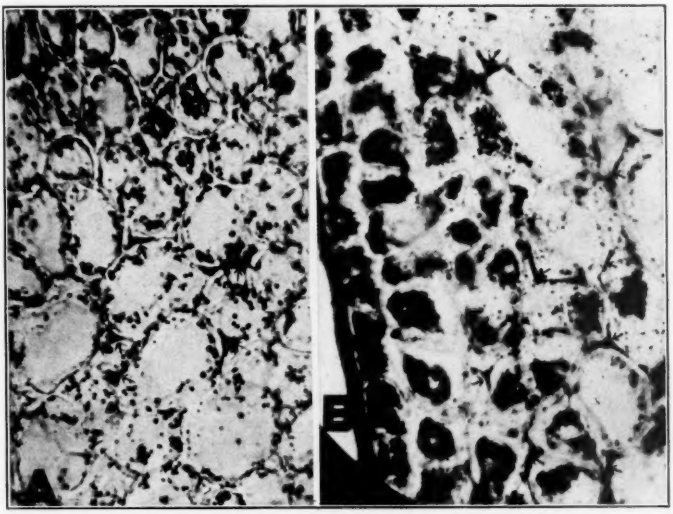

FIG. 9. *A*, section of orange rind showing spray oil 10 days after spraying with Oronite Technical oil; *B*, section of rind from lemon which had shown considerable browning of albedo. Oil was found on side not showing browning of albedo as well as on browned side. This lemon had been through coloring process of packing house.

In no case was there any indication of oil conduction from the leaves into the twig. In some instances the oil had apparently gone 1 or 2 cm. along the stem by capillarity, but no oil which could be identified as spray oil was found in the conductive tissues in any case.

This experiment was repeated, using oils dyed with oil red O, but again no evidences of oil conduction were found.

HISTOLOGICAL INDICATIONS OF INJURY

Sections were made from orange and lemon trees which had been sprayed 2 years previously with undiluted oils varying in viscosity from

30 to 288 seconds and varying in unsulphonatable residue from 98 to 50 per cent.

Those sprayed with oils containing from 70 to 50 per cent. of unsulphonatable residue showed severe injury in the form of breakdown of the cells in the region of the cambium, leaving a ring of large cavities at least part of the way around the twig (fig. 10, *A* and *B*). These cavities were filled with gum and often tyloses extended out into them from the surrounding cells. The new wood which had been laid down after the spray had been applied was wavy and knurled in appearance.

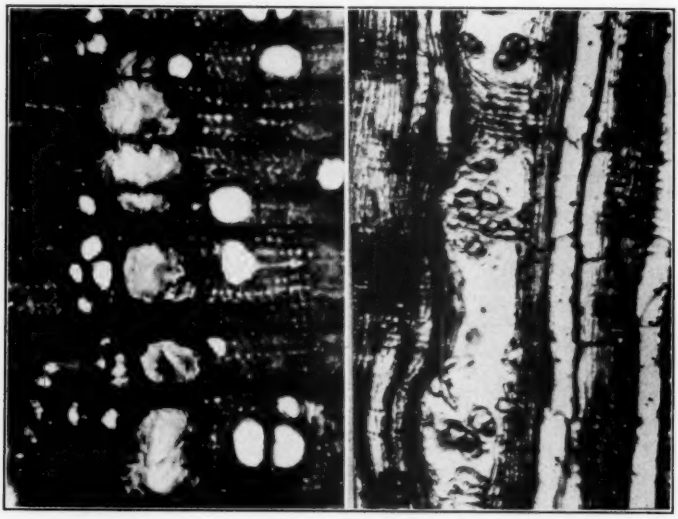

FIG. 10. Breakdown of cells caused by unsulphonatable oils: *A*, cross-section of lemon twig; *B*, longitudinal section of lemon stem.

Sections of stems from trees sprayed with oils of more than 70 per cent. unsulphonatable residues did not show these cavities, but practically all showed some irregular or abnormal cells in that region which had been cambium or primary xylem at the time the spray was applied. These cells appeared wavy or corrugated in longitudinal sections. The walls of the wood fibers in this region were usually thinner than those of normal ones.

Sections of twigs of trees sprayed with undiluted 100-viscosity oil containing 85 per cent. of unsulphonatable residue showed a pronounced wavy appearance. They also contained considerable oil in the wood fibers. No histological indications of injury have been observed when commercial emulsions have been used.

Petroleum oil within the cells of the plant

Petroleum oil has not been identified in the living cells of the plant such as the chlorophyll-bearing cells of the leaf and the cells of the cortex, phloem, and medullary rays of the stem, although it sometimes appears that an occasional cell of the living part of the leaf or stem has petroleum oil in it. Cells containing large oxalate crystals frequently contain spray oil. If a freezing microtome is used there is much less evidence of oil in any living cells.

The oil enters the wood fibers and often completely fills many of them, but this is only in stems which have been sprayed or painted with undiluted oil or whenever enough oil has been applied so that it penetrates the woody cylinder.

There are also evidences that at times the oil gets into the xylem tubes, but this happens only when the stem is practically saturated with oil. Oil has never been found in the xylem tubes of commercially sprayed trees.

Penetration of spray emulsion, as such, into leaf and stem

Kelly (**10**) has indicated that the spray emulsion enters the leaf of the apple. There is no indication that this is true with citrus leaves. If citrus leaves are placed in water under reduced pressure, the air is removed, and when the pressure is again brought to normal the leaf fills with water. This is readily noticeable by the water-soaked appearance of the leaf. Such appearances do not normally occur in leaves sprayed with oil emulsions. The oil-soaked areas mentioned in this paper resemble to some extent the water soaking, except that the oil soaking occurs along the midrib and along the margin, and when the heavier oils are used it is permanent throughout the life of the leaf.

To determine more fully whether water would enter the leaves of citrus, orange twigs including the leaves were sprayed with solutions of different water-soluble dyes. After the leaves had dried, the twigs were cut and taken to the laboratory. Sections of the leaves and twigs were made but no traces of the dyes could be found. The dyes used were acid fuchsin, trypan blue, thionin, malachite green, and safranin O. In these tests no emulsifier was used. It was omitted because the absence of an emulsifier usually increases the oil-soaked appearance when applying oil sprays.

In sections of leaves which had been sprayed with emulsions, whether tight or quick-breaking emulsions, the oil showed the same relative distribution through the leaf as when undiluted oils were applied.

If, as Kelly (**10**) has stated, the oil enters the plant in the form of an emulsion, the globules should be found scattered throughout the mesophyll and other tissues having intercellular spaces. It is certain that the oil could

get no farther than into the intercellular spaces of the leaf as an emulsion, for it would have to pass through a cell wall in order to get into the conduction tract of the plant. In passing through a cell wall it would have to go through as a solution or as a much finer emulsion than is common in these sprays, at least as long as the cell was alive. There seems to be no significant difference in the appearance of the oil which has entered through the upper surface where there are no stomata and that which enters through the stomata of the lower surface of the leaf, except that the oil is more in films than in globules, because of the fact that the cells of the palisade layers are much closer together. As long as the oil is in the palisade region it has little of the globular appearance. When sufficient oil is added so that it goes beyond the palisade layers into the spongy parenchyma, it takes on the same globular appearance that it has when it enters from the lower side through the stomata. This is probably due to the fact that it has more room to spread out and form globules.

While observations point to the conclusion that the oil enters the plant almost entirely as a continuous oil film drawn in by capillarity, it is difficult to say with any certainty that the force which pulls the oil into the plant tissue is capillarity. If the walls of the cells in the leaf and in the stem are wet on the outer side, it is difficult to explain this movement by capillarity, since capillarity depends upon the cohesion of the liquid to the surface of the capillaries. If the surface of the cell walls is covered with a lipoid film, or some other surface to which the oil could cohere, then capillarity might well be the explanation of the movement of the oil into the tissue.

The section shown in figure 3 *C*, which was stained with dye in a water medium, shows clearly that the masses of oil are rounded on the ends and do not appear to be adhering to the cell walls. This, however, may be due to the water which has been added to the section. There is nothing to indicate that the intercellular spaces are filled with water emulsion by spraying. They do not have the appearance of being water-soaked, except perhaps along the outer edge and along the midrib. This water-soaked appearance is evidently not due to water, since it stays for at least some weeks and sometimes years; whereas if it were due to water it would disappear rapidly. Sections of the leaves indicate that this water-soaked appearance is due to oil between the cells of the palisade layers.

Physical effect of oil around the cells of the plant

The question arises as to whether some of the cells of the plant, especially in the oil-soaked area of the leaf, are completely surrounded by oil. Cross-sections of leaves through the oil-soaked parts of the palisade layers and sections cut parallel to the dorsal surface through these areas appeared

as though some of the palisade cells were completely surrounded. It is certain that if these cells are not completely surrounded they at least have their exposed areas greatly reduced. If these cells are completely surrounded they must have some protoplasmic connections with those cells which are not surrounded, or there must be an adequate interchange of mineral solutes and gases through the oil film, for there is no evidence of any dead cells. However this may be, at least a small number of citrus leaves remain on the trees for as long as two or more years after being sprayed, without showing any ill effects from the oil.

In this connection it is recognized that an oil layer many times the thickness of the films (which are usually not more and generally are much less than 4 μ thick) around these cells is necessary in order to produce anaerobic conditions. It is also recognized that oils should not always be considered toxic to all kinds of protoplasm. DUGGAR (**7**), MELHUS and DURRELL (**14**), and GOLDSWORTHY and SMITH (**9**) have shown that petroleum products stimulate the growth of some fungus spores. CONN (**3**) has shown that white mineral oil, vaseline, and paraffin produce a marked stimulation of the growth of *Streptococcus*. CONTOLEON and JOANNIDES (**4**) found no demonstrable effect of liquid petrolatum in retarding bacterial growth *in vitro* or in the intestines. BAILEY (**1**) has kept cambium initial cells alive and actively streaming for 500 hours (about 21 days) in White Russian oil. MCWHORTER (**13**), however, has reported that Volck Concentrate is a good control for mildew on roses.

Quantitative determination of petroleum spray oils in citrus leaves and twigs

Several investigators have worked on quantitative methods for measuring the spray oil retained by leaves and other surfaces. SMITH (**17**) has described methods for weighing the oil deposited on glass slides and on leaves by various oil emulsions. ENGLISH (**8**) has described a method for measuring the oil retained by citrus leaves. This method consists of cutting fifty discs, each 10 sq. cm. in area, from sprayed leaves and extracting them by shaking them twice for 1 minute, using 50 cc. of ethyl ether each time. ENGLISH's method has been tried out in this laboratory, and after shaking the leaves with ether for as long as 30 minutes, it was found that not all of the petroleum oil was removed from them. This was determined by drying the leaves and then extracting them again with petroleum ether and determining the oil content as described in this paper. ENGLISH's method, however, may give results which are relative to the actual quantity of oil present in the leaf.

It is shown in figure 3 *B* of this paper that the oil is not evenly distributed throughout the leaf, but is concentrated along the midrib and espe-

cially near the margin. It is therefore believed that the use of the discs from the leaves does not give a representative sample of the oil deposit.

DAWSEY and HAAS (5) have recently described another method for measuring the quantity of petroleum spray oil deposited on camphor, pecan, and orange leaves. This method of extracting the oil from the camphor and pecan leaves is similar to the method used by ENGLISH (8). These investigators also experienced some difficulty in extracting all of the oil from citrus leaves, and they have somewhat modified ENGLISH'S method.

PREPARATION OF MATERIAL

Two methods were used in the selection of samples of leaves for extraction. In most of these tests the samples were obtained by picking a given number of leaves from each of several trees (usually three to five trees were used), and usually not fewer than 500 leaves were used for the total sample. A definite number of leaves was selected from each of the four sides of a tree. Only healthy, medium-sized leaves were collected. As soon as the samples of leaves were picked they were placed in paper bags and taken to the laboratory where they were dried. Two methods of drying were used. The samples used in the tests recorded in table VI and in figures 11 and 12 were placed in an oven to dry at a temperature not to exceed 80° C. In all other data given, the samples were dried in wire trays at room temperature. As soon as the samples were dry they were ground to powder in a Wiley mill, using a screen having 1-mm. holes. This ground material was then placed in tight tin containers until such time as the extractions could be made.

In collecting samples from commercially sprayed groves a different method was used. The samples were obtained by collecting two branches from opposite sides of each of a number of trees. These branches were then tied together, the bundle tagged, and taken to the laboratory where the leaves were picked, dried, and ground. If the trees from which these samples were collected had put out new leaves which had grown to maturity so that they could not be distinguished from the sprayed leaves, the twigs were left until the leaves wilted and then only those leaves which showed some oil-soaked area were picked.

Samples of twigs were obtained in the same manner as were the leaves from commercial groves. After these twigs had been brought to the laboratory and the leaves picked off, the twigs under 0.25 inch in diameter were cut in pieces about 6 inches long. They were then ground in the Wiley mill and placed in tin containers.

SOLVENTS USED IN EXTRACTING OIL

Acetone, benzene, ethyl ether, and petroleum ether were tried as solvents. Since the petroleum oil must be separated from the oily or waxy

materials extracted from the plant tissues, it is desirable to have an extract that is as nearly free as possible of the latter materials. Petroleum ether is a good solvent for the petroleum oils, and since it extracted much less of the plant materials than did the other solvents, it proved to be satisfactory for this work. There are probably no two lots of petroleum ether which will be found alike even though they may be labeled b.p. 30° to 60° C. Some lots have been obtained which are much less volatile. It was because of this that it was found necessary to run a stream of air into the flasks as described later. The ether obtained from the Central Scientific Company of Chicago has given satisfactory results, while ether obtained from one other source has not been suitable for this work.

Methods of extracting and measuring spray oil

Twenty-five gm. of each of four samples of ground leaves or twigs were weighed out and placed in four Erlenmeyer flasks of 300 cc. capacity. About 175 cc. of petroleum ether (boiling point 30° to 60° C.) were added to each flask. The flasks were placed on the table for 30 minutes and shaken at intervals of 5 minutes during this time. The extract was filtered from the plant tissue by using a specially made Buchner funnel. The sides of the cup part of this funnel are 12 cm. high and the inside diameter of the cup is 9 cm. The contents of the extraction flask can be emptied into this funnel rapidly with little danger of loss. The funnel was placed on a suction flask and the solvent and extract were drawn through (not too rapidly) by means of reduced pressure. The plant material was then washed several times with petroleum ether, these washings being added to the extract. The original filtrate was then returned to the original flask. The four flasks of total filtrate were placed in a constant temperature water bath at 60° to 65° C. and each connected to a condenser for recovering the ether as it distilled off. When the ether had distilled down to about 5 cc. or less, the flasks were disconnected from the condenser and a stream of air from the compressed air line was run into the flasks by means of a small glass tube inserted into the flasks. The stream of air was allowed to run slowly against the bottom of the flasks for approximately 10 minutes, or longer if there was a large quantity of plant extract present. The air aided greatly in driving off the last traces of ether.

When all of the ether had been driven off, 5 cc. of sulphuric acid of specific gravity 1.840 to 1.835 were added to each flask. The flasks were then allowed to remain in the water bath for 5 minutes, after which 5 to 10 cc. more of acid were added. The flasks were rolled around so that the acid came in contact with any material on the sides of the flasks. They were then returned to the water bath for another 5 to 10 minutes. Best results were obtained by avoiding any hard shaking or beating, as this

tended to break the oil into very fine globules, making it difficult to bring the oil to the surface of the acid when centrifuged.

As soon as all plant material was digested, and when no black undigested material was left adhering to the bottom or sides of the flasks, the contents were transferred to Babcock milk test bottles by means of a small glass funnel, the stem of which had been drawn out to a 2-mm. opening. The flasks were then rinsed several times with 5 to 10-cc. portions of acid until the test bottles were filled. The bottles were filled only to the bottom of the graduated column, as expansion due to heating in the centrifuge ran the bottles over if they were filled too full.

When all four samples were in the test bottles they were placed in a high-speed centrifuge which was equipped with a thermostatically controlled heating unit, keeping the temperature between 60° and 70° C. This heating unit was composed of a 1000-watt heating coil from an electric stove. It was fastened to the under side of the centrifuge lid. A De Khotinsky thermoregulator was inserted through a hole drilled in the top of the centrifuge. This heater raised the temperature in the centrifuge to 65° C. in about 10 minutes and the temperature seldom varied more than 2°. The speed of the centrifuge was about 1200–1300 r.p.m. Each set of samples was centrifuged for not less than 30 minutes after the centrifuge had become heated. After centrifuging, the test bottles were removed and the length of the oil column in each was measured.

In most of the work reported here, Babcock bottles which had the same length of graduated neck were used. The graduations were not used, but a vernier caliper was used to measure the length of the oil column. The readings were recorded in millimeters and later converted to cubic centimeters. The graduated necks of the Babcock milk test bottles hold 1.58 cc. and are divided into 80 divisions; thus each division has a capacity of 0.0197 cc. The number of these divisions that are filled with oil after centrifuging when multiplied by the factor 0.0197 gives the number of cubic centimeters of oil in a given sample. For example: If a 25-gm. sample of plant material yielded 12.5 divisions of oil, then $12.5 \times 0.0197 = 0.246$ cc. of oil per 25 gm. of plant material, or 0.984 cc. per 100 gm. of plant material. This method of measuring the oil in the Babcock bottles may be found more satisfactory than measuring the oil column with the vernier caliper.

The data given in table II are the results of tests to determine whether an appreciable loss of the lighter oils would result from the treatment given them in the extraction process. These data indicate that about 2 per cent. of the lighter oils (such as Mineral Seal) will be lost when heated on a water bath at 65° C. for 70 minutes. Mineral Seal oil with a viscosity of 50 was used.

The volatile portion of such oils probably disappears during the dry-

TABLE II

RATE OF DISTILLATION OF PETROLEUM ETHER FROM MINERAL SEAL OIL AND AMOUNT OF LOSS OF OIL DURING EVAPORATION OF ETHER

TEST	WT. OF OIL IN FLASK	WT. AFTER BEING AT ROOM TEMPERATURE			WT. AFTER BEING IN WATER BATH 70 MIN. AT 65° C.	LOSS
		5 HR.	24 HR.	46 HR.		
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.641	1.641	1.637	1.637	1.607	0.034
2	1.644	1.647	1.644	1.642	1.608	0.036

ing of the test samples; therefore the oil obtained in the extraction of dried leaves is probably not entirely accurate. The loss of oil from drying the leaves is probably very small from samples of leaves collected more than 10 days after the spray has been applied, because the more volatile portion of the oil has already volatilized while the leaves were on the tree.

In order to determine the length of time necessary to drive all of the ether from the flasks, samples of dry, ground leaf tissue weighing 29 gm. each were extracted. This material had not been sprayed and therefore it had no spray oil in it. The ether was then driven off in the manner already described. When the material had come to a constant weight, approximately 0.2 cc. of Mineral Seal oil was pipetted into the extract in each flask. The flasks and contents were again weighed. Ether was added and the

TABLE III

RATE OF DISTILLATION OF PETROLEUM ETHER FROM MIXTURES OF PLANT EXTRACT AND MINERAL SEAL OIL

SAMPLE NO.	WT. OF PLANT EXTRACT	WT. OF OIL ADDED	HEATED AT 65° C.				
			10 MIN.	20 MIN.	30 MIN.	40 MIN.	50 MIN.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.263	0.160	+ 0.435	+ 0.181	+ 0.014	− 0.006	− 0.009
2	0.472	0.188	+ 0.494	+ 0.185	+ 0.023	− 0.005	− 0.008
3	0.292	0.194	+ 0.509	+ 0.181	+ 0.043	− 0.005	− 0.007
4	0.294	0.184	+ 0.469	+ 0.183	+ 0.033	− 0.005	− 0.007

contents of the flasks dissolved. The flasks were again placed in the water bath and the ether distilled off at 65° C. During this process the flasks were taken out and weighed at each 10-minute interval. The weight of

extract, the weight of oil added, and the weight above or below the original weight before the ether was added, are given in table III.

At the end of 40 minutes all the flasks showed a very small loss in weight, indicating that all the ether had distilled over by that time.

TABLE IV

COMPARISON OF DISTILLATION RATE OF PETROLEUM ETHER AND ETHYL ETHER FROM PETROLEUM OIL

SOLVENT	WT. OF OIL ADDED	WEIGHT AFTER HEATING		
		10 MIN.	20 MIN.	30 MIN.
	gm.	*gm.*	*gm.*	*gm.*
P. E.	0.176	+0.439	+0.205	+0.005
P. E.	0.169	+0.452	+0.151	−0.001
E. E.	0.172	+0.471	+0.137	−0.000
E. E.	0.177	+0.485	+0.124	−0.003

In order to determine whether it was more difficult to free the oil from petroleum ether than from ethyl ether, a similar test was made but without the leaf extract, using two samples with petroleum ether and two others with ethyl ether. These were all run simultaneously at a temperature of 58° C. The results are given in table IV. These results indicate that with this lot of petroleum ether there is no significant difference in the rate at which the two ethers are distilled from the oil.

The results of fourteen oil determinations from one large sample of leaves taken from a commercially sprayed grove are given in table V. This material was collected three months after application of the spray. The tests were made to determine the accuracy of the extraction method. These tests of twelve samples were each extracted for 20 minutes. They show a variation in the oil obtained ranging from 0.458 to 0.496 cc. per 100 gm. of dry tissue. The average for the twelve samples was 0.477 cc. The two samples extracted for 24 hours show a little more oil, 0.496 and 0.544 cc. respectively. This indicates that not quite all of the oil was removed from the leaves in 20 minutes.

RATE OF DISAPPEARANCE OF VARIOUS SPRAY OILS FROM LEAVES OF CITRUS

For this experiment five oils were used: Oronite Technical, Oronite X, Special 80, Oronite Cosmetic, and Oronite Mineral Seal, with specifications as given previously. Five large Valencia trees were sprayed with each oil, using the tank-mix method with a casein spreader and 2 per cent. oil.

TABLE V

OIL OBTAINED FROM SAMPLES OF VALENCIA ORANGE LEAVES SPRAYED COMMERCIALLY THREE MONTHS PREVIOUSLY

SAMPLE NO.	OIL IN 50 GM. LEAVES	OIL PER 100 GM. LEAVES	TIME EXTRACTED
	mm.	*cc.*	*min.*
1	10.1	0.486	20
2	10.1	0.486	20
3	9.8	0.470	20
4	10.3	0.496	20
5	9.5	0.458	20
6	9.5	0.458	20
7	10.1	0.486	20
8	10.0	0.482	20
			hrs.
9	10.3	0.496	24
10	11.3	0.544	24
			min.
11	9.7	0.468	20
12	9.9	0.476	20
13	10.0	0.482	20
14	10.0	0.482	20

Samples of leaves were collected from these trees at intervals and the oil content determined by the method already described. The results of these tests are given in table VI (graphically represented in fig. 11). This table gives the length of the column of oil in the neck of the test bottle in millimeters for each of the two tests of each sample. These tests show that practically all of the Mineral Seal oil had disappeared in 173 days while about 33 per cent. of the heavy Oronite Technical oil remained in the leaves.

These data (table VI, fig. 11) indicate that there is a rapid disappearance of all oils for the first three weeks. They show that the disappearance of oil after the first three weeks is relatively slow, and that there is still an appreciable quantity of the heavier oil in the leaves nearly six months after the application.

Collections from these same trees were made in which the material from the north side of the trees was kept separate from that on the south. The data are given graphically in figure 12.

It is evident from these data that the oil disappears more rapidly from the leaves on the south side of the tree than from those on the north side. There seems to be little if any difference in the rate of disappearance of the oil from the twigs on the two sides of the tree. These data also fail to show

TABLE VI

PETROLEUM OIL FOUND IN VALENCIA ORANGE LEAVES SPRAYED SEPTEMBER 8, 1930, WITH DIFFERENT OILS
LEAVES DRIED IN OVEN AT 80° C. WITH EXCEPTION OF LAST TWO COLLECTIONS WHICH WERE DRIED AT ROOM TEMPERATURE
25-GM. SAMPLES USED IN EACH TEST

DAYS AFTER SPRAY APPLICATION	ORONITE MINERAL SEAL		ORONITE COSMETIC		SPECIAL 80		ORONITE X		ORONITE TECHNICAL	
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1	SAMPLE NO. 2
	*mm.**	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	14.2	12.7	22.2	23.3	17.6	16.5	25.5	24.2	25.0	25.1
4	8.0	8.6	20.0	19.4	13.9	14.0	20.5	20.5	21.5	20.3
10	5.0	6.0	14.5	14.8	10.5	10.5				
15	4.3	4.4	11.7	12.8	9.1		15.0	15.3	17.0	17.5
22	3.7	3.5	11.6	11.5	7.2	7.2	12.8	11.6	15.7	14.5
39	3.6	3.2	11.0	11.7	5.8	6.1	10.8	11.5	15.2	
55	2.3		8.0	8.4	5.0	5.4	9.9	10.9	14.5	13.7
84	2.0	2.2	5.0	5.5	4.5	4.3	9.8	9.7	12.9	13.3
94	2.2	2.4	7.7	7.8	5.3	5.1	8.2	9.1	12.3	12.8
173	Trace		5.0		3.0		6.5	6.4	8.0	8.0

* mm. = length in mm. of oil column in Babcock milk test bottle.

any evidence that the disappearance of the oil from the leaves was due to a movement of the oil into the twigs.

In order to be more certain of the results in table I, another set of similar tests was run. Five navel orange trees were sprayed with each of the following sprays: 2 per cent. Oronite Technical Mixol; 2 per cent. Oronite

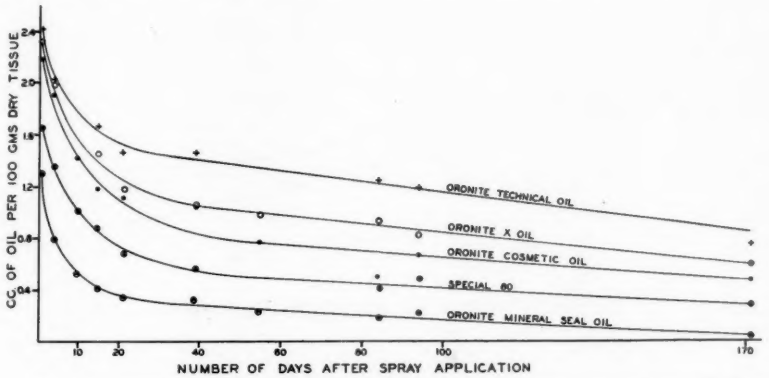

FIG. 11. Graph showing rate of disappearance of petroleum spray oil from Valencia orange leaves sprayed with 2 per cent. oil in tank-mix emulsion Sept. 9, 1930, to Feb. 28, 1931. Leaves dried at 80° C.

Technical tank-mix, using 4 ounces of blood albumin spreader per 100 gallons of spray; 2 per cent. Volck Concentrate; 2 per cent. Volck Medium; 2 per cent. Volck Light; 2 per cent. Oronite Mineral Seal tank-mix, using

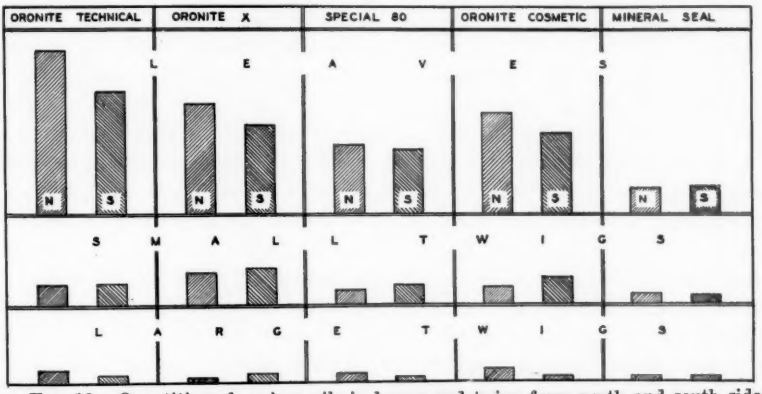

FIG. 12. Quantities of various oils in leaves and twigs from north and south sides of orange trees sprayed Sept. 8, 1930. Collections made Dec. 12, 1930.

4 ounces of blood albumin spreader per 100 gallons of spray. Five trees were left as checks. Samples of leaves were collected at intervals and dried in the laboratory at room temperature. The results of the tests are shown graphically in figure 13.

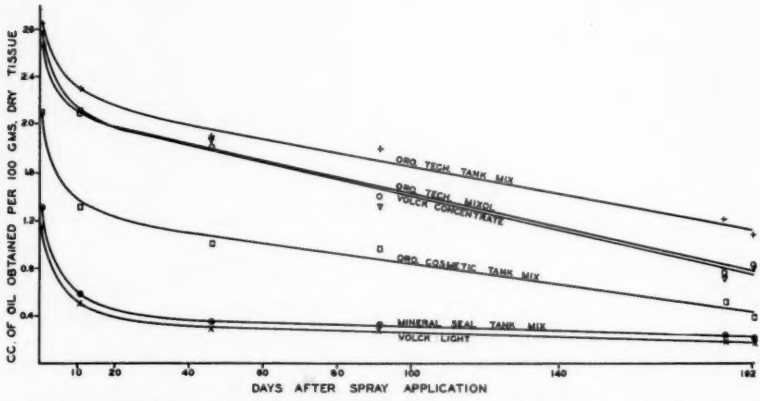

FIG. 13. Graph showing rate of disappearance of petroleum spray oil from navel orange leaves sprayed with 2 per cent. oil emulsions July 27, 1932, to Jan. 26, 1933. Leaves dried at room temperature.

The graphs in figure 13 show results remarkably similar to those in figure 11 except that the curves in figure 13 indicate that the oil did not disappear so rapidly during the first three weeks. This is no doubt due to the fact that the leaves used for the data given in figure 11 were dried in an oven at 80° C., while those used for figure 13 were dried at room temperature. It will be noticed also that the curves in figure 13 show that the rate of disappearance of Volck Concentrate and Oronite Technical Mixol is somewhat more rapid than that of the Oronite Technical tank-mix. After 192 days the Volck Concentrate and the Oronite Technical Mixol show 0.75 cc. of oil per 100 gm. of dried leaves, while the Oronite Technical tank-mix shows about 1.12 cc. of oil per 100 gm. of dried leaves (or about 50 per cent. more oil). It is believed that this slower disappearance of the tank-mix emulsion may be due to a difference in the size of the oil globules. The difference in size of oil globules may be caused by a higher surface tension in the tank-mix, which results in a smaller amount of oil surface exposed to the air in those tissues sprayed with tank-mix emulsions. Therefore tank-mix emulsions may have a slower evaporation rate.

Table VII gives the results of tests made on materials collected from commercial groves which had been sprayed with widely different materials

TABLE VII

OIL FOUND IN CITRUS LEAVES COLLECTED FROM COMMERCIAL GROVES

GROVE NO.	LOCATION	DATE SPRAYED (1931)	TIME AFTER APPLICATION	SPRAY MATERIAL	KIND OF TREES	DEPT. AGR. OIL GROUP NO.*	OIL PER 100 GM. LEAVES
			months				*cc.*
1	Whittier	Oct. 22	10	Volck Light Medium 1–2/3%	Valencia	2	0.28
2	Orange	Oct. 1	11	Standard 65 T.M.	"	2	Trace
3	Orange	Sept. 20	12	Orthol K. Lt. Med.	"	2	0.21
4	Whittier	Aug. 31	12	Volck Lt. Med. 2%	"	2	0.19
5	Riverside	July 12	12	Medona 65	Navel	2	0.19
6	"	July 22	12	Medona 65	"	2	0.22
7	"	July 26	12	Orange-ol	"	2	0.15
8	Orange	Oct. 31	10	Volck Med. T.M.	Valencia	3	0.33
9	Whittier	Sept. 17	11	Volck Med. 2%	"	3	0.25
10	Orange	Sept. 31	11	Volck Med. T.M.	"	3	0.32
11	Whittier	Aug. 31	12	Volck Med. 2%	"	3	0.33
12	Orange	Oct. 31	12	Volck Med. 2%	"	3	0.33
13	Whittier	Sept. 17	11	Volck Conc. 2%	Lemon	5	0.64
14	"	Sept. 18	11	Lemon Mixol 3%	"	5	0.62
15	"	Sept. 18	11	Del Monte Heavy 2%	"	5	0.57
16	"	Sept. 31	11	Volck Conc. 2%	"	5	0.68

* Classification of California State Department of Agriculture based on distillation:
51 to 63 per cent. of no. 2 oil distills over 636° F.
43 to 51 per cent. of no. 3 oil distills over 636° F.
0 to 29 per cent. of no. 5 oil distills over 636° F.

at different times and under different conditions. These data indicate that usually there is a small quantity of oil still on the trees at the end of one year in commercially sprayed groves, even when the lighter oils are used. There is a distinct difference in the quantities of oil left on the trees by the oils in the different groups, but within any one group the variation is less than might have been expected.

Figure 14 gives graphically some of the results of an experiment designed to determine whether there was any difference in the type of pene-

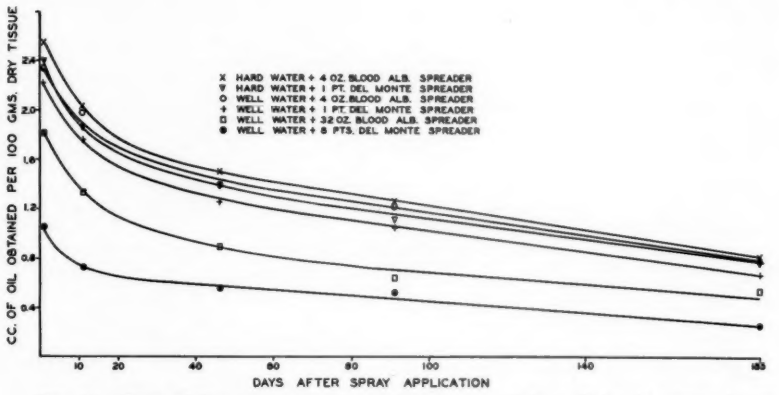

FIG. 14. Rate of disappearance of petroleum spray oil from Valencia orange leaves sprayed with 2 per cent. Oronite Technical oil July 27, 1932, to Jan. 26, 1933.

tration where widely differing quantities of spreader are used and where hard and moderately soft water are used in the emulsions.

Four large Valencia orange trees were sprayed with each of the emulsions used. Two per cent. of Oronite Technical oil was used in all of these sprays.

Two types of emulsifiers were used. The blood albumin spreader is recommended by the University of California (17) for use in the tank-mix emulsions. Four ounces of spreader per 100 gallons of water is the recommended amount. The other spreader used was the Del Monte spreader which is put out by the California Spray Chemical Corporation. This is a liquid spreader and it is recommended for use at the rate of 1 pint per 100 gallons of water. For the soft water the Riverside city water was used. This water has a conductivity of about $K \times 10^5 = 40$, or equal to about 0.0037 normal $CaCl_2$ (equivalent to 103 gm. $CaCl_2 \cdot 2H_2O$ per 100 gallons of water). This is rather soft water in comparison with most waters in southern California. For the hard water the same water with the addition of 537 gm.

of $CaCl_2 \cdot 2H_2O$ per 100 gallons was used. The addition of this salt brought the conductivity $K \times 10^5$ to about 225, which is about as hard as any water which would be used in southern California. These data indicate that the added "hardness" had some effect in causing a heavier deposit of oil by both emulsifiers, but the difference is not great.

The emulsions in which blood albumin was used deposited more oil than those in which the Del Monte spreader was used. This may have been due to the fact that the blood albumin used had been on hand for several months, and its solubility may have been less than that of fresh spreader. The emulsions in which eight times the normal quantity of spreader was used showed a marked reduction in the quantity of oil deposited. The one in which the Del Monte spreader was used deposited only about one-third as much oil as some of the other sprays. There is also a wide difference in the quantity of oil deposited by the 32 ounces of blood albumin and by the 8 pints of Del Monte spreader, the blood albumin depositing nearly twice as much oil.

Sections from leaves sprayed with the different emulsions showed no significant differences in the appearance of the oil in the tissues nor in its general location. There was considerable difference in quantity of oil-soaked area present, this difference being in accord with the results of the extractions. The oil-soaked areas in the leaves were limited to only very small places here and there on the edges of those leaves which were sprayed with emulsions containing an excessive quantity of spreader, while the others contained extensive oil-soaked areas sometimes covering nearly half of the leaf surface.

Comparison of results of histological studies with results obtained by oil extraction

Little can be determined by histological studies as to the quantity of oil in the tissues, since the distribution of the oil is so uneven throughout the different parts of the tree and even throughout the different parts of the same leaf or twig. In the more open parts of the plant, such as the spongy parenchyma of the leaf, the oil is more or less in large globules, while in the palisade tissues it is in very thin films between the closely fitting cells. It is difficult to compare under the microscope the quantities of oil in the two places.

The extraction of oils does not give much information as to the exact location of the oil, but it does give good indications as to the quantity present in any given tissue. Sometimes one may extract leaves which have been sprayed for as long as a year or more previously, and find measurable quantities of oil. It may be difficult to find oil in sections of these leaves

unless one allows the leaves to wilt, after which one may pick out those showing oil-soaked areas. Stained sections of these areas will show the presence of oil. Again, one may find a few leaves with small oil-soaked areas which when sectioned through the oil-soaked parts will show the presence of oil, but there may be such a small total area containing oil that in extracting only a trace of it will be found.

Summary and conclusions

1. A method is presented for the staining of sections of leaves, stems, and fruit of citrus trees by means of which the natural plant oils may be distinguished from the petroleum spray oils which have been applied to the trees.

2. The path of entrance and the location of the spray oils in the different tissues are discussed and illustrated.

3. A quantitative method for purifying and measuring the petroleum spray oil extracted from citrus tissues is described. This method has been used for the determination of the rate of disappearance of the petroleum oil from the trees.

4. The spray oils appear to enter the plant tissues by capillarity. When applied according to recognized spray practice the oil does not penetrate deeply into the plant tissue. It remains between the cells just under the epidermis of leaves, stems, and fruit. Only in the case of heavy applications does it penetrate to a depth of more than half a dozen plant cells beneath the epidermis. Under the usual conditions of application, no evidence has been found that the oil enters those plant cells which contain protoplasm. The less volatile portions of all except the lightest of the spray oils remain in the citrus leaves throughout their lifetime, often more than two years. In spite of the oil in these leaves they appear to remain healthy.

5. The petroleum oils are not found to be distributed evenly in the leaves, but are concentrated along the midrib and especially along the margin. Their distribution may be seen by the oil-soaked areas in leaves which have been allowed to wilt.

6. When sufficient quantities of oil are applied to citrus, the oil may penetrate deeply, even to the pith of twigs several centimeters in diameter.

7. No evidence has been found of any translocation or other movement of oils from leaves into twigs or from small twigs into larger twigs, except as the oils may migrate short distances between the cells by capillarity.

8. Extraction and measurement of the spray oil in citrus leaves indicate that with the heavier oils about one-third of the oil disappears during

the first three weeks after application, while with the lighter oils two-thirds to three-fourths of the oil may disappear during this time.

9. The data presented indicate that there is but little of a lighter spray oil left on a tree six months after its application, while as much an one-third of a heavier oil may be present after that length of time.

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CARBOHYDRATE–NITROGEN RATIOS WITH RESPECT TO THE SEXUAL EXPRESSION OF HEMP

PAUL J. TALLEY

(WITH TWO FIGURES)

Introduction

Most of the recent work on the determination of sex in the higher plants has been done by cytologists and geneticists. These investigations have resulted in the chromosome theory of sex inheritance and sex determination, which is supported by much evidence in the dioecious forms of the higher plants.

Any adequate theory of sex inheritance and determination in the angiosperms must do more than account for the usual approximate 1:1 ratio of the sexes in dioecious plants. It must also explain the reversal of sexes within the so-called dioecious plants and the determination and development of both types of sporophylls on the same plant, as in the monoecious types. It should even explain the development of both types of sporophylls within one flower, as is the case with the majority of the angiosperms. The segregation of the microsporophylls and the macrosporophylls to individual plants should be considered the exception and not the general case. Any theory of sex determination based only on dioecious types is subject to many exceptions and criticisms.

The absence, or apparent absence, of allosomes in many dioecious plants and the frequency of reversal from the staminate to the pistillate condition, or *vice versa,* in forms in which allosomes have been reported, have led to differences of opinion as to the final importance of the "sex chromosomes" in sex determination in the dioecious angiosperms. The reversal of the sexes in dioecious plants indicates that sex determination and inheritance may result from the action of two different, but closely related, sets of factors in the development of these plants. The prevailing 1:1 ratio of the sexes indicates that a genetical tendency to produce one or the other type of flower is probably inherited in a strictly Mendelian manner. The evidence from the development of intersexes under certain environmental conditions shows clearly that the final display of the staminate or of the pistillate condition is determined by physiological as well as by genetic factors, even in dioecious plants. Probably the development of staminate and pistillate flowers is more dependent on physiological factors in monoecious than in dioecious plants. In plants with perfect flowers the development of stamens and pistils is probably chiefly dependent on physiological factors. Indeed it would be difficult to explain the determination of the

sex of the floral organs in monoecious plants and in plants with bisexual flowers on only a Mendelian basis, although genetic factors undoubtedly play an important part in determining the order of appearance and the arrangement of stamens and pistils in bisexual flowers and of pistillate and staminate flowers in monoecious species.

If the chromosome theory of sex inheritance is accepted for dioecious plants, it need not imply that sex determination is finally accomplished by means of the chromatin content alone. The observations in nature indicate that the potentialities for both types of spores are present at all times in all heterosporous angiosperm sporophytes, whether dioecious, monoecious, or perfect with respect to flower types. This has frequently been assumed. In that case dioecism would be considered as the suppression of one type of sporophyll on a given plant and monoecism would imply the suppression of one type of sporophyll only within a given region of the floral structures. The inherent nature to behave in this manner, under the usual conditions of growth, is probably controlled by genetic factors. Whether or not this is the case, there remains the need for an explanation of the controlling internal physiological mechanism that is functioning at the time of determination of staminate and pistillate structures. This internal physiological mechanism is at present the unknown factor. Regardless of our lack of knowledge concerning the nature of this physiological mechanism, it has been assumed to be the fluctuating factor which makes possible the reversal of the sexes in dioecious plants. This same concept can be applied to the development of only one type of fertile sporophylls in one region and of the other type of fertile sporophylls in another region of the same heterosporous plant. The physiological mechanism in question need not be fixed; perhaps it may be changed by an altered environment or by the metabolism of the plant.

Geneticists in general do not attempt to explain the mechanism by which a gene, or set of genes, brings about the manifestation of the particular morphological and physiological characters with which they are connected, but this manifestation is thought to be the result of a series of processes which begins with the interaction between the chromosome substance and the particular type and state of the cytoplasm within which the chromosomes are contained. Many genes are recognized which are influenced, or limited, in their actions by the conditions of the plant or of the tissue in which the character is expressed. If this is the case for genes influencing sexual expression, then it is obviously necessary to postulate a difference in the physiological conditions varying with time or location, or both, which makes possible the expression of the staminate or pistillate conditions in the ordinary monoecious plant or in a plant with perfect flowers. The genetic constitution is not known to be different in the stami-

nate and pistillate organs of these plants, with the possible exception of *Begonia* (**20**). Thus there is need for a study of the physiological differences associated with the development of stamens and pistils to explain the determination of sex in the higher plants. It is obviously difficult to obtain data which are conclusive in this respect. It will ultimately be necessary to determine whether or not the physiological differences represent the changes which accompany or follow the real determining physiological factors.

The problem of the physiological factors associated with the formation of staminate and pistillate structures respectively has not been directly attacked. Obviously the difficulties in making such a study on plants with bisporangiate flowers are very great. The problem should be less difficult in such monoecious plants as corn, and least difficult in a dioecious plant like hemp.

Review of literature

KLEBS (**11**) emphasized the complexity of the factors influencing the types of structures formed by plants. He considered the living cell as being influenced by three sets of factors: (1) the specific structure, (2) the internal conditions, and (3) the external conditions. His use of the term specific structure includes what might be termed the inherent composition of the cell. By varying the environmental factors in his studies on *Saprolegnia mixta* (**11**) and *Sempervivum funkii* (**10**), KLEBS showed clearly that the behavior of these organisms is not controlled by a fixed inner rhythm but is subject to control (within limits) by external factors.

Since the work of KLEBS, the most important advances in the mechanics of development and in our knowdge of the factors determining differentiation in the seed plants have fallen along two general lines. KRAUS and KRAYBILL (**12**) report a correlation between the carbohydrate and nitrogen content of the tomato plant and fruitfulness. The literature on carbohydrate-nitrogen ratios has become so extensive that no attempt will be made to review it here. The writer knows of no work pertaining to carbohydrate-nitrogen ratios in the two sexes of a dioecious angiosperm.

Another line of advance in our knowledge of the factors determining growth and reproduction in the angiosperms was initiated by GARNER and ALLARD (**6**) in their work on photoperiodicity. Probably the reaction of the plant to the proper photoperiod is only one means of producing an internal condition favorable to the formation of flowers and of influencing sexual reproduction. It should be possible to produce this same end result by altering one or more factors in the environment of the plant other than the photoperiod.

Some photoperiodic studies have been conducted on dioecious plants.

SCHAFFNER (**23, 25**), MCPHEE (**15**), and ADAMS (**1**) have varied the daily period of illumination on hemp. SCHAFFNER found that a decreased length of day is accompanied by an earlier flowering and an increase in the relative number of sex reversals. He reported that almost 100 per cent. of the staminate plants show some pistillate structures when flowering occurs in the middle of the winter. MCPHEE failed to obtain as high a percentage of reversals or intersexes as SCHAFFNER reported. ADAMS gave no data on this phase of the subject.

WESTER (**29**), in an account of the work of CIESIELSKY on sex determination in hemp, referred to the latter's conclusion that sex is normally determined by the age of the pollen at the time of pollination, but BESSEY (**2**) and BOSE (**3**) failed to find any support for this theory from their experiments on pollen age and sex.

The cytological aspect of sex inheritance in hemp has been studied by several workers. STRASBURGER (**28**) and MCPHEE (**14**) failed to find any apparent sex chromosomes, but SINOTÔ (**26**) and HIRATA (**9**) reported allosomes from studies on meiosis in staminate plants.

The genetical aspect of sex inheritance in hemp has been studied in breeding experiments by MCPHEE (**16**) and HIRATA (**8**). MCPHEE suggested that the genetical mechanism is of the XY type, the staminate plants being heterozygous for the factor or factors involved. The conclusions of MCPHEE are supported by the work of HIRATA.

It is generally agreed that the sexual expression of hemp can be modified in certain strains by environmental factors. MCPHEE (**16**), HIRATA (**7**), and BOSE (**3**) have discussed the frequency of sex reversals in different strains of hemp. CORRENS (**5**) has classified hemp as trioecious because the appearance of monoecious plants is so frequent in this species.

SCHAFFNER (**24**) has caused hemp plants to pass from a vegetative condition into a flowering condition and then back to a vegetative state for a prolonged period of time by changing the photoperiod. When flowering recurs the staminate plants frequently produce some intersexes. BOSE's (**3**) observations show that the reversals of sex are more common in the staminate plants. He found that the occurrence of intergrades in the field is limited almost completely to the flowers formed at the beginning or near the end of the period of flowering. These two observations support the view that conditions existing within the plant largely influence the types of flowers found at a given time. If the determining factors are physiological, one might expect that the conditions within the plant determining the formation of staminate flowers, or inhibiting the formation of pistillate flowers, on a genetically staminate plant would be gradually developed. One need not assume that these internal physiological factors remain fixed from the time of initiation of the first flower primordia until the cessation of flower formation.

PRITCHARD (**21**) obtained sex reversals in hemp by mutilating the plants, that is, by removing the floral branches or leaves. Covering the tips of the plants with manila bags was found to be equally effective in this respect. The injection of solutions of carbohydrates and nitrogenous compounds into the stem after mutilation seems to be of further help in causing the production of intersexes in some cases. BOSE (**3**) attempted to repeat this work, using the transpiration stream as a means of distributing the liquids through the plant instead of injecting them mechanically. Both investigators found that decapitation and covering the tips with bags are the best means of forcing sex reversals in the field. BOSE doubted the significance of his results since some intersexes appeared in his controls.

The metabolic differences between the sexes in dioecious angiosperms have not been investigated to any extent. SATINA and BLAKESLEE (**22**) applied several biochemical tests to certain Mucorales in an effort to distinguish metabolic differences between the heterothallic strains of this group of fungi. They applied similar tests to the leaves of some of the dioecious angiosperms, hemp being among those tested. They found that the Manoilov reaction, which is based on the reduction of potassium permanganate, is of a different order for the leaves of staminate and pistillate plants of hemp. They also reported a difference between the sexes in the natural color of the alcoholic extracts and in the oxygenase and the peroxidase activities, while the pH of the two types of plants is practically the same. They used only the leaves from staminate and pistillate plants. CAMP (**4**) reported that the catalase activity of reproductive organs, or structures closely associated with them, is higher in the case of staminate tissues than in the corresponding tissues of pistillate plants of the same species. The same relationship was reported by him for the catalase activity of the staminate structures or flowers in contrast to activity of the pistillate structures or flowers of monoecious plants or plants with bisexual flowers.

Many indications of physiological differences between staminate and pistillate angiosperms exist. Such differences are frequently assumed to be important in sex determination, but there is a noticeable paucity of data on the subject. The results here presented are the outcome of an attempt to ascertain some of the differences and similarities in staminate and pistillate plants of hemp.

Methods

Hemp seeds obtained from a commercial seed company were planted in soil trays on February 7 and transplanted into a deep soil bed in the greenhouse on February 22. The length of the photoperiod was increased to 15 hours by means of a battery of electric lights. A few male plants were beginning to flower on March 19. On April 2 typical male and

typical female plants were selected for analyses. The most mature flowers in the inflorescences of the staminate plants were beginning to shed pollen, but most of the flowers on each plant were immature at this time. A few of the flowers on the pistillate plants were fully developed, but no seed had been set. The average heights were 40 cm. for the pistillate plants and 52 cm. for the staminate plants. On April 5 another collection was made. These plants were in essentially the same stages of development as those collected three days earlier.

The plants were cut at the cotyledonary node at 4 P. M. and placed in separate weighed flasks for the determination of their fresh weights. Each plant was then cut into small pieces. The macerated tissue was mixed thoroughly and divided into two portions, each of which was weighed.

One of the portions was used for the determination of the percentage of dry matter in the sample and subsequently employed for the estimation of nitrogen. The other portion of each plant was used for the determination of carbohydrates. It was placed in 80 per cent. alcohol with a slight excess of calcium carbonate and refluxed for one hour to complete the extraction of the soluble carbohydrates. The procedure given by LOOMIS (**13**) was followed for the separation of the soluble and insoluble carbohydrates. All extracts were cleared by the use of natural lead acetate and deleaded by the use of potassium oxalate. Tests for reducing sugars were made on aliquots of the extracts obtained in this manner. Total sugars were determined on aliquots of these extracts after they had been hydrolyzed with 2 per cent. hydrochloric acid at 70° C. for 10 minutes and then neutralized with sodium carbonate.

That portion of the sample of each plant which was insoluble in 80 per cent. alcohol was hydrolyzed with 2 per cent. hydrochloric acid at the temperature of boiling water for two hours. The solution was then neutralized, cleared, and deleaded. Aliquots of this liquid were used for reducing sugar tests to estimate the acid-hydrolyzable polysaccharides present in each plant.

The carbohydrate fractions were determined by the use of Fehling's solution. The cuprous oxide was estimated by the volumetric iodide method given by MAHIN and CARR (**17**). Determinations were made on duplicate aliquots of each fraction of the carbohydrates. Each class of carbohydrates was expressed in terms of its glucose equivalent.

The nitrogen content of that portion of each plant used for dry weight determinations was estimated by the Kjeldahl method. Digestion was accomplished by the use of 20 cc. of concentrated sulphuric acid and 0.5 gm. of cupric sulphate. The distillate was received in normal acid and titrated with normal sodium hydroxide using methyl red as an indicator.

Results

The determinations of the dry weights show no differences between the percentages of moisture present in the aerial portions of the staminate and pistillate plants. There is a marked difference in the content of reducing sugars in the plants of the two sexes. The average content of reducing sugars expressed in terms of the fresh weight of the plants is 0.56 per cent. for the male plants and 0.23 per cent. for the female plants in the collection made on April 2. The same values for the collection made on April 5 are 0.81 for the males and 0.27 for the females. This striking difference still exists for the collections of both dates if the values are computed in terms of the dry matter. The differences in the contents of total sugars, expressed as glucose, also show the males to be higher than the females in the total quantity of the more soluble carbohydrates present on both dates. The results of these determinations are given in columns 2–6 inclusive of tables I and II.

The acid-hydrolyzable polysaccharides, expressed in terms of glucose, are more abundant on the average in the males than in the females, but the content of polysaccharides varies so much within the staminate plants that the average value for the group loses its possible significance (see columns 8 and 9 of tables I and II). The contents of the various classes of carbohydrates in the staminate and pistillate plants collected on April 5 are presented graphically in figure 1.

The average content of nitrogen is higher in the females than in the males, whether the values are computed on the basis of fresh weight or on the basis of dry matter. The results of the determinations of nitrogen are given in columns 12 and 13 of tables I and II.

The differences in the contents of the various carbohydrate fractions and of the nitrogen present in the staminate and pistillate plants are accentuated when the results are expressed in terms of the ratios of the carbohydrates to the nitrogen. The ratios of the reducing sugars to the nitrogen are higher for the staminate than for the pistillate plants. The same relationship holds for the ratios of the total sugars to the nitrogen. The average values of the ratios of the polysaccharides to the nitrogen are higher for the males than for the females; however, a few males have lower values than some few of the females for this ratio. The ratios of the various groups of carbohydrates and of the total carbohydrates to the nitrogen for the plants of both sexes are given in table III. The ratios for the plants collected on April 5 are presented graphically in figure 2.

Discussion

The determinations of the content of dry matter fail to show any differences between the sexes. If differences between the compositions of the

TABLE I

FRESH WEIGHTS AND PERCENTAGES OF DRY MATTER AND MOISTURE AND AMOUNTS OF REDUCING SUGARS, TOTAL SUGARS, POLYSACCHARIDES, TOTAL CARBOHYDRATES, AND NITROGEN AS PERCENTAGES OF FRESH AND DRY WEIGHTS OF STAMINATE AND PISTILLATE HEMP PLANTS HARVESTED APRIL 2

	Plant no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	13.1	21.03	78.97	0.79	3.78	1.38	6.58	3.01	14.3	4.39	20.9	0.81	3.88
2	11.3	20.81	79.19	0.48	2.34	1.43	6.90	3.01	14.5	4.44	21.4	0.80	3.84
3	15.8	20.86	79.14	0.43	2.07	1.24	5.94	2.78	13.3	4.02	19.2	0.90	4.29
4	5.7	20.59	79.41	0.47	2.25	2.13	10.27	2.81	13.6	4.94	23.9	0.85	4.12
5	3.4	24.49	75.51	0.40	1.62	3.47	14.05	5.70	23.1	9.17	37.2	0.77	3.11
6	17.6	21.12	78.88	0.78	3.68	1.45	6.88	2.75	13.0	4.20	19.9	0.67	3.16
Average	11.2	21.48	78.52	0.56	2.62	1.85	8.44	3.34	15.3	5.19	23.7	0.80	3.73
						Pistillate plants							
1	14.0	21.43	78.57	0.20	0.94	1.15	5.33	2.96	13.8	4.11	19.1	0.99	4.63
2	15.0	20.55	79.45	0.20	0.98	1.05	5.12	2.27	11.0	3.32	16.1	0.90	4.36
3	17.6	20.57	79.43	0.22	1.09	1.15	5.60	2.94	14.3	4.09	19.9	0.95	4.63
4	12.8	21.16	78.84	0.22	1.02	1.09	5.14	2.98	14.1	4.07	19.2	0.95	4.48
5	20.5	21.28	78.72	0.25	1.18	0.99	4.64	2.71	12.7	3.70	17.3	0.90	4.25
6	13.5	18.77	81.23	0.27	1.46	0.90	4.83	2.44	13.0	3.34	17.8	0.88	4.71
Average	15.5	20.63	79.37	0.23	1.11	1.06	5.11	2.71	13.2	3.77	18.3	0.93	4.51

TABLE II

FRESH WEIGHTS AND PERCENTAGES OF DRY MATTER AND MOISTURE AND AMOUNTS OF REDUCING SUGARS, TOTAL SUGARS, POLYSACCHARIDES, TOTAL CARBOHYDRATES, AND NITROGEN AS PERCENTAGES OF FRESH AND DRY WEIGHTS OF STAMINATE AND PISTILLATE HEMP PLANTS HARVESTED APRIL 5

	Plant no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Total fresh weight	Dry matter	Moisture content	Reducing sugars		Total sugars		Polysaccharides		Total carbohydrates		Nitrogen	
				Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis	Fresh weight basis	Dry weight basis
	gm.	%	%	%	%	%	%	%	%	%	%	%	%
						Staminate plants							
1	9.4	23.27	76.73	0.78	3.36	1.94	8.34	2.62	11.2	4.56	19.5	1.04	4.47
2	8.8	22.42	77.58	0.76	3.39	1.49	6.64	2.68	12.0	4.17	18.6	0.88	3.92
3	8.5	23.11	76.89	0.80	3.45	1.81	7.83	3.81	16.5	5.62	24.3	1.04	4.52
4	6.0	24.17	75.83	1.02	4.19	2.66	10.94	3.89	16.0	6.55	26.9	1.02	4.22
5	28.2	20.13	79.87	0.36	1.76	1.28	6.33	2.22	11.0	3.50	17.3	0.93	4.64
6	7.1	23.78	76.22	1.12	4.68	1.95	8.18	3.39	14.3	5.34	22.5	0.84	3.52
Average	11.4	22.81	77.19	0.81	3.47	1.86	8.04	3.10	13.5	4.96	21.5	0.96	4.22
						Pistillate plants							
1	16.7	23.98	76.02	0.26	1.08	1.17	4.88	2.63	11.0	3.80	15.9	1.12	4.68
2	21.7	17.18	82.82	0.20	1.16	0.99	5.78	1.88	10.9	2.87	16.7	0.92	5.33
3	9.2	25.31	74.69	0.31	1.21	1.21	4.77	3.09	12.2	4.30	17.0	1.22	4.82
4	18.5	20.52	79.48	0.26	1.28	0.97	4.74	2.10	10.3	3.07	15.0	1.00	4.87
5	19.0	20.97	79.03	0.34	1.63	1.09	5.19	2.37	11.3	3.46	16.5	0.98	4.70
6	25.5	23.11	76.89	0.23	1.01	1.13	4.90	2.68	11.5	3.80	16.4	1.12	4.82
Average	18.4	21.85	78.15	0.27	1.23	1.09	5.04	2.46	11.2	3.55	16.3	1.06	4.87

sexes exist, they are to be found in the compositions of the dry matter and not in the percentages of dry matter.

The analyses show several striking differences in the compositions of the dry matter of the sexes. The average content of carbohydrates is higher in the males than in the females. The significance of the average values is decreased by the fact that the lowest values for individual staminate plants

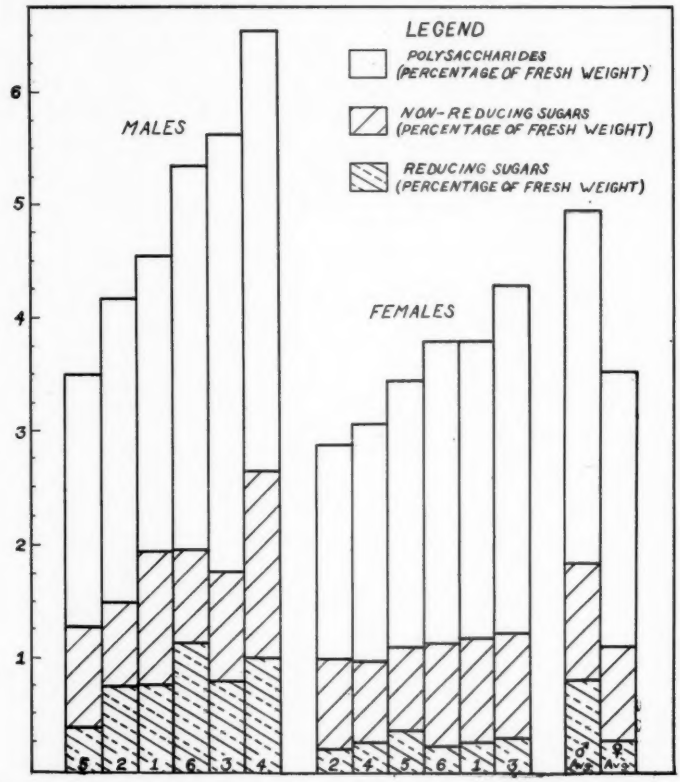

FIG. 1. Polygons showing carbohydrate contents of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

are less than the highest values for a few of the pistillate plants. Tables I and II show that in this case one is not only comparing staminate and pistillate plants but is also contrasting the largest staminate plants and the smallest pistillate plants. Usually the pistillate plants have a much greater total fresh weight than the staminate plants at the time of flowering. There are

TABLE III

RATIOS OF CARBOHYDRATE FRACTIONS TO THE NITROGEN

PLANT NO.	REDUCING SUGARS/ NITROGEN	TOTAL SUGARS/ NITROGEN	POLYSACCHARIDES/ NITROGEN	TOTAL CARBOHYDRATES/ NITROGEN
Staminate (April 2)				
1	0.98	1.70	3.70	5.40
2	0.60	1.79	3.77	5.56
3	0.48	1.38	3.10	4.48
4	0.55	2.50	3.31	5.81
5	0.52	4.51	7.42	11.93
6	1.16	2.17	4.11	6.28
Average	0.71	2.34	4.24	6.58
Pistillate (April 2)				
1	0.20	1.16	2.99	4.15
2	0.22	1.17	2.52	3.69
3	0.23	1.21	3.09	4.30
4	0.23	1.15	3.14	4.29
5	0.28	1.09	2.99	4.08
6	0.31	1.02	2.76	3.78
Average	0.25	1.14	2.91	4.05
Staminate (April 5)				
1	0.75	1.86	2.51	4.37
2	0.86	1.69	3.05	4.74
3	0.77	1.74	3.66	5.40
4	1.00	2.60	3.80	6.40
5	0.38	1.37	2.38	3.75
6	1.33	2.32	4.05	6.37
Average	0.85	1.93	3.24	5.17
Pistillate (April 5)				
1	0.23	1.04	2.35	3.39
2	0.22	1.09	2.05	3.14
3	0.25	0.99	2.53	3.52
4	0.26	0.97	2.10	3.07
5	0.35	1.11	2.41	3.52
6	0.21	1.02	2.39	3.41
Average	0.25	1.04	2.31	3.34

individual exceptions to this rule which probably can be accounted for in part by the lack of genetical uniformity in the seeds.

In contrast to the percentages of total carbohydrates, the percentages of total sugars not only show a higher average value for the staminate

plants in contrast to the average value for the pistillate plants, but there is no overlapping in the quantities of carbohydrates soluble in 80 per cent. alcohol present in the individual plants of the two sexes. The lowest value for any of the males is 1.24 per cent. of the fresh weight, while 1.21 is the highest percentage for any one female plant. This difference in the quantities of total sugars in the plants of the opposite sexes is found to be due almost entirely to the quantities of reducing sugars. This class of carbohydrates

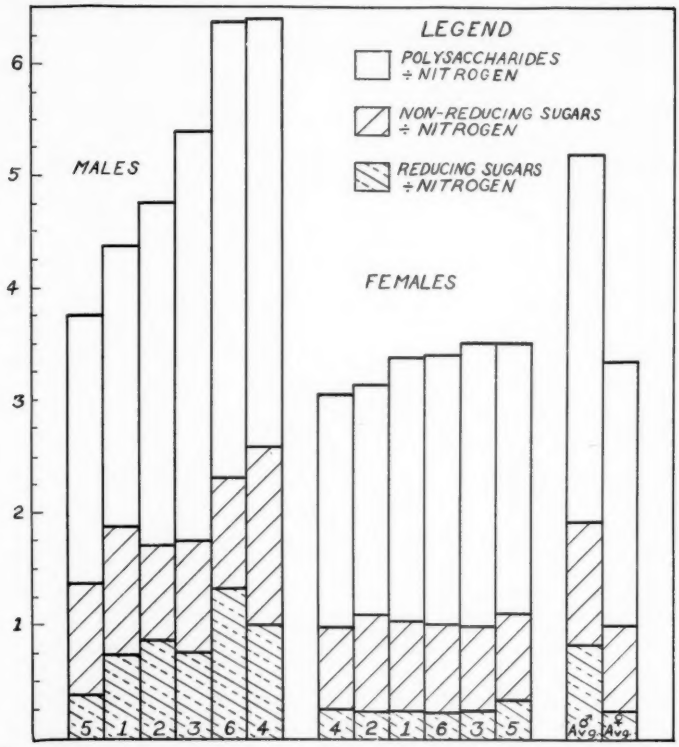

FIG. 2. Polygons showing carbohydrate-nitrogen ratios of staminate and pistillate plants of hemp harvested April 5. The number in the base of each polygon refers to the experimental number for the plant.

presents the most contrasting difference between the chemical compositions of the sexes of hemp under the conditions in which these plants were grown. The average values for the reducing sugars, expressed as percentages of the fresh weight, are from two to three times as high for the males as for the females. The ranges of variation within the two sexes cover two separate

regions. Although there is a difference in the quantities of total sugars in the two sexes, it is based chiefly on the differences in the reducing sugars and not on the quantities of non-reducing sugars. Figure 1 presents these facts graphically and also indicates the ranges of variability in the contents of carbohydrates of the two sexes.

The pistillate plants are relatively constant; that is, the analyses show little variation in the percentages of reducing and total sugars in these plants. The situation existing within the staminate plants is different. The quantities of reducing and total sugars fluctuate from values slightly greater than those for the highest pistillate plants up to values clearly of a different order.

The higher content of carbohydrates and the wider range of fluctuation in all classes of carbohydrates in the staminate plants, in contrast to the condition in the pistillate plants, are very suggestive. One is inclined to consider that the presence of the greater quantities of reducing and total sugars in the males is not a result of the inability to synthesize polysaccharides, since they too are usually more abundant in the staminate plants. Nor is one justified by these analyses alone in assuming that the polysaccharides have been digested more rapidly in the males than in the females. Regardless of the causes of the differences in the quantities of the various fractions of carbohydrates in the sexes, the constancy of the females is in keeping with the later behavior of the plants. The females flower more gradually and apparently retain their vegetative vigor for a longer period of time than do the males. The staminate plants usually turn yellow and die rapidly after flowering. The greater variability in the content of carbohydrates in the males and their early senescence stand in contrast to the small amount of variability in the carbohydrates and the slow loss of vegetative vigor in the females, when both sexes are growing under early spring conditions in the greenhouse.

The results of the nitrogen analyses are not so significant. The pistillate plants have a larger average percentage of their fresh weights composed of nitrogen than have the staminate plants. However, the values for the individual staminate and pistillate plants do not fall into two groups as in the case of the reducing sugars. It is not advisable to attempt any detailed evaluation of the nitrogen determinations, since they include all of the reduced forms of nitrogen within the plants. It is probable that most of this nitrogen is present in the plant in complex organic molecules.

The ratios of the various classes of carbohydrates to the nitrogen present some interesting differences between the sexes (table III and fig. 2). The ratios of the reducing sugars to the nitrogen again show the females to be less variable than the males. The ratios are much greater for the staminate plants than for the pistillate plants; the average values for the males

are about three times as great as are those for the females. The greater ratios of the males are due chiefly to their higher content of reducing sugars but are partly the result of their lower content of nitrogen. This same situation exists in the ratios of the total sugars to the nitrogen, but the differences between these ratios for the sexes are not so great. It is evident that the main differences in the ratios of the total sugars to the nitrogen are chiefly the result of the differences in the ratios of the reducing sugars to the nitrogen. The constancy of the females is again evident in these ratios, while the males continue to show a greater variability.

The ratios of the polysaccharides to the nitrogen show that the males have a higher average ratio than the females, but the ranges of variability in these two groups overlap. These ratios obviously are not so significant as the ratios of the sugars to the nitrogen in differentiating between the sexes of hemp grown under the conditions of these experiments. The combinations of these ratios for the different plants, that is, the ratios of the total carbohydrates to the nitrogen, give higher average values for the staminate plants than for the pistillate plants. The lowest ratio for a staminate plant collected on April 2 is higher than the highest value for any pistillate plant collected on that date. The same relationship exists for the ratios of the staminate and pistillate plants collected on April 5. The ratios are greater for the plants collected on April 2 than they are for the collection of April 5. This is partly the result of a lower nitrogen content in the plants of both sexes collected on April 2 and a lower content of polysaccharides in the plants collected on April 5.

From the preceding discussion it is evident that the contents of carbohydrates and nitrogen are different in staminate and pistillate hemp plants at the time of flowering, when they are grown under the conditions of these experiments. These differences are seen to be the result of a relatively high content of sugars and a relatively low content of nitrogen in the staminate plants. These facts can be interpreted to explain the early senescence of the staminate plants and the retained vegetative vigor of the pistillate plants of greenhouse hemp. KRAUS and KRAYBILL (**12**) and others have shown that the relationships between the carbohydrates and nitrogenous materials are largely the determining factors in changing from the vegetative to the fruiting state in several bisporangiate angiosperms. The results obtained in these analyses of hemp plants in the early stages of flowering do not necessarily show any relationship to the quantities of these substances which were present before the plants began flowering, but it is evident that these staminate and pistillate plants have different carbohydrate-nitrogen ratios at the time of flowering. It remains to be determined whether the differences between the sexes in these ratios are causal physiological factors connected with the formation of stamens and pistils, or

whether they are merely accompanying factors. It does not necessarily follow that the conditions in the meristematic regions of the staminate and pistillate plants at the time of flower formation are properly reflected by the analyses of the whole aerial portions of the plants.

MURNEEK (**18**) has shown that tomato plants grown with a shortage of nitrogen turn yellow and decline vegetatively. This condition can soon be overcome by furnishing the plant available nitrogenous compounds. The staminate hemp plants, soon after they flower, act similarly to the nitrogen-starved tomato plants. A difference exists chiefly in the fact that the staminate hemp plants continue to decline even though they have the same source of nitrogen compounds as have the pistillate plants. They are apparently exhausted by the flowering process to the extent that they are unable to continue the assimilation of nitrogen rapidly enough to regain their lost vegetative vigor, as does the tomato plant.

The spider flower (*Cleome spinosa*) yields some interesting information with respect to types of flowers formed and the quantity of nutritive materials present. STOUT (**27**) has described the development of alternating whorls of fertile and sterile flowers in this bisporangiate angiosperm. MURNEEK (**19**) has altered the nitrogen available to this plant and finds intermittent sterility in plants with an abundance of or with a shortage of available nitrogen in the soil. MURNEEK found a difference in the growth of the plants and in the frequency of the alternating whorls of sterile and fertile flowers, but he was unable to cause the plant to overcome the tendency to form alternately fertile and sterile flowers by changing the quantity of nitrogen available to its roots. The removal of flowers containing fertile pistils has a direct effect on the type of flowers produced in the meristematic region of the spike. The percentage of the flowers able to set seed was increased greatly by this procedure. The intermittent sterility in the spider flower results from the alternation of whorls of flowers with aborted stamens and large functional pistils with whorls of flowers containing aborted pistils and large functional stamens. Thus the functional sex of the flowers of *Cleome spinosa* varies with some internal regulating condition determining the development of the essential floral parts. Since the removal of fertile flowers and fruits increases the width of the zones of fertile flowers being formed nearer the apex of the inflorescence, it seems that the formation of functionally pistillate flowers and the maturation of fruits both require similar, or the same, substances from the plant. Either these substances are not present in sufficient quantities or else they are not conducted to all parts of the inflorescence rapidly enough to permit a continuous formation of fertile flowers and the simultaneous maturation of fruits.

Since the removal of fruits is known to allow the nitrogenous compounds

to accumulate in certain plants (**18**), it is probable that the fertility of the flowers produced by *Cleome spinosa* is partly dependent upon the nitrogen supply available in the tissues in close proximity to the flower primordia. If this is true for the spider flower, one might consider that the functionally staminate flowers of *Cleome spinosa* were formed from meristematic regions lower in certain nitrogenous elements, or with a higher carbohydrate-nitrogen ratio, than the regions forming functionally pistillate flowers. The condition in hemp plants might be essentially the same except that the usual presence of aborted stamens or pistils within the flowers is not encountered. Since these analyses include the whole aerial portions, it is not safe to assume positively that the formation of stamens takes place in tissue with a higher carbohydrate-nitrogen ratio, while pistils are formed in a corresponding tissue with a lower carbohydrate-nitrogen ratio. If this were definitely proved, it would not necessarily follow that the sexual nature of the flower depended entirely on the quantities of these metabolic materials; but it is interesting to note this possible relationship between the sexual nature of the plant and the quantities of sugars and nitrogenous compounds present.

A further investigation into other aspects of the physiological differences and similarities between the sexes in hemp and in other plants is needed before one can properly evaluate the true significance of the carbohydrate-nitrogen ratios with respect to sex determination.

Summary

1. The aerial portions of staminate and pistillate plants of hemp were analyzed at the time they were coming into flower. Determinations were made of the total fresh weight of the individual plants and of the moisture, reducing sugars, total sugars, polysaccharides, and reduced forms of nitrogen.

2. Little if any difference in the percentages of moisture and dry matter exists between the sexes of hemp.

3. Staminate plants have higher average percentages of total carbohydrates, polysaccharides, and sugars than pistillate plants under the conditions of these experiments.

4. The reducing sugars are much more abundant in the staminate plants than in the pistillate plants.

5. Nitrogen is relatively more abundant in the pistillate plants than in the staminate plants.

6. The carbohydrate-nitrogen ratios of the sexes are discussed with respect to the differences in the sexual expressions and the habits of growth of the plants.

Acknowledgment is made of the encouragement and suggestions given by Professor W. F. LOEHWING of the University of Iowa during the early part of this investigation. Appreciation is expressed to Professor J. B. OVERTON of the University of Wisconsin, for his kind criticisms and advice in the preparation of this paper.

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LOSSES TO THE CORN CROP CAUSED BY LEAF INJURY

GEORGE H. DUNGAN

(WITH SIX FIGURES)

Introduction

The corn plant as it is grown in the corn belt is subject (1) to accidental injuries by man and his implements; (2) to destruction of tissues by insects and rodents; and (3) to rough treatment by nature in the tearing and twisting by strong winds, and in the beating and bruising action of rain and hail. This report deals with losses due to mutilations of the type caused by hailstorms.

Unfortunately hail does not injure certain rows of corn and leave the adjoining rows uninjured so that losses can be accurately compared and measured. Definite information of this kind can be obtained only from planned experiments. It is difficult if not impossible, however, to treat corn plants artificially in a way that accurately duplicates the injury caused by natural hailstorms. Notwithstanding the element of inaccuracy, experiments of the type here described are believed to be the best means of extending our knowledge of the degree of functional derangement due to injuries of a definite type and severity.

Nature of injury caused by hail

Hailstorms damage small corn plants mainly by shredding and beating off the blades. Large hailstones, if they strike small plants near the surface of the soil, may break them at the ground level.

With large plants hail causes injury by splitting the blades and by tearing away entire blades or portions of blades. Hailstones also bruise the stalks with a consequent interference with the movement of materials within the plant (fig. 1). If the hail occurs at pollination time, it may seriously interfere with normal fertilization of the kernels through the destruction of the functioning organs of the tassels and silks. Storms later in the season may result in direct damage to the developing ears. These may be knocked from the plant by large hailstones striking the supporting shank, or more frequently the corn on the cob may be bruised through the husk. Sometimes the impact is so great as to mash the immature kernels. Molds usually follow such injuries, especially if the ear is in the blister or milk stage.

Probably the greatest loss occasioned by hailstorms comes from injury to the leaves. Losses from blade injury are more difficult to estimate than

losses due to ear bruising. For this reason the experiments reported here were confined almost entirely to the leaf-injury and blade-removal phases of the problem.

According to a summary of the reports made by crop correspondents of the U. S. Department of Agriculture, the annual loss to the corn crop

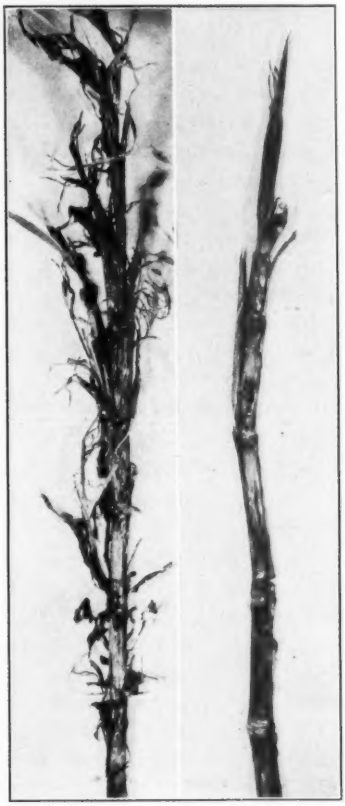

FIG. 1. Corn plants injured by severe natural hailstorm on July 22, 1931. Most of the emerging tassels were either destroyed or so badly injured that they could not function in pollen production. Blades and sheathes have been cut away from plant on right to show the hailstone bruises on the stalk.

caused by hail in the United States averaged 0.6 per cent. during the period 1909–1924 (**14**). Even though this is a small percentage loss, it is considerable when expressed in bushels. This average loss of 0.6 per cent. is the result of a relatively few farmers suffering severe losses, sometimes approaching 100 per cent.

VALGREN (**15**) shows that the geographic division of the country including Illinois, Indiana, Michigan, Ohio, and Wisconsin suffered, as a 10-year average for 1909–1918, the smallest reduction in corn yield due to hail of any similar sized section of the United States. Although this reduction amounts to only 0.25 per cent. of the total crop, or 2,800,000 bushels, nevertheless, taken from a few growers, it represents a serious loss.

Experiments of other investigators

In some localities it is, or used to be, a common practice to cut off the corn plant just above the ear and use the cured tops for live-stock feeding. Topping is occasionally accompanied with stripping of the blades from that portion of the stalk below the ear. Stripping is sometimes practiced without topping. In order to obtain the tops and blades when they are high in feeding value, they are usually taken from the plant when the grain reaches the glaze or early dent stage.

CONNELL (**1**), TRACY and LLOYD (**13**), NEWMAN (**9**), and HUNT (**8**) found that the removal of green blades by such practices as topping and stripping was associated with a reduced yield of grain and frequently this loss in yield of corn was not compensated for by the value of the forage secured.

DIBBLE and MARSTON (**3**) topped corn to determine the value of this method as a means of controlling the European corn borer. As an average of tests covering two seasons, removing the tops just above the ear was followed by a 39 per cent. reduction in yield of grain. Topping 10 to 12 inches above the ear resulted in a 12 per cent. drop in yield, and topping 20 to 24 inches above the ear lowered the yield 5 per cent.

SAYRE, MORRIS, and RICHEY (**11**) found that the reduction of the leaf area of corn plants was associated with a decrease in the total sugar content of the stalk.

STEGGERDA (**12**) found that corn plants which had been injured produced less grain than uninjured plants, and the reduction in yield increased with the increasing number of blades removed.

CULPEPPER and MAGOON (**2**), DUNGAN (**4, 5**), ELDREDGE (**6**), and HUME and FRANZKE (**7**) found that the removal of all blades from corn plants at tasseling time produced the greatest reduction in yield. The greatest injury to the quality of grain resulted from defoliations made just after the silks had turned brown. Splitting and shredding the blades and breaking the midribs, or otherwise mutilating the blades if they were not entirely severed from the plant, did not result in such great yield reductions as did leaf pruning. It seemed that no matter into how many shreds the blades were torn, as long as the parts were attached to the plant they were capable of functioning in grain formation.

Methods

In the first artificial hailing experiments at Illinois, the hail-like injury was inflicted with a whip made of a bundle of 20-inch lengths of baling wire. By striking the blades and stalks with the end of this whip, a wound resembling both a bruise and a tear was produced.

Since it was believed that the main source of injury from hail was probably the reduced blade area, most of the later treatments involved leaf

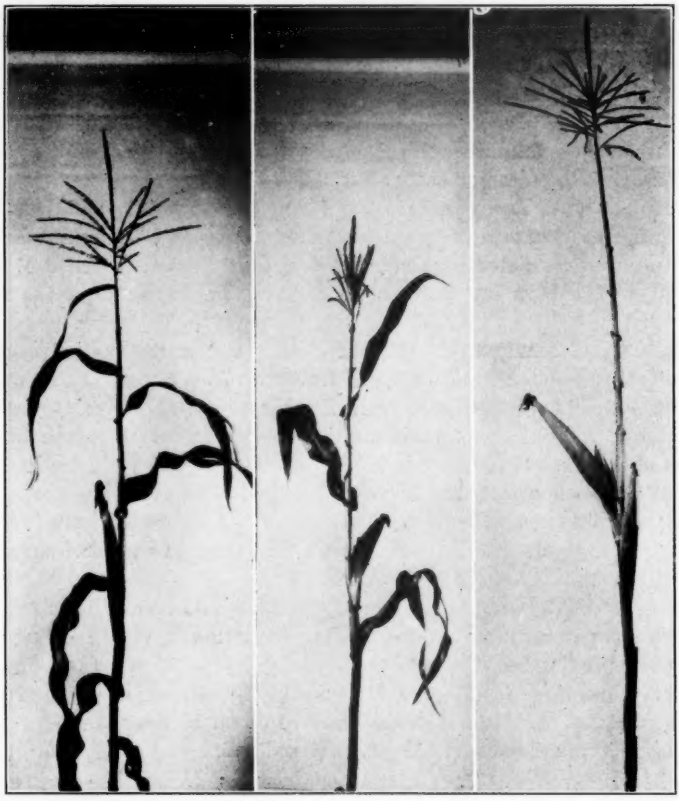

FIG. 2. Plants from which three, eight, and all blades were removed with hand shears.

removal, either by jerking the blade from the sheath by hand, or by clipping them off with hand shears (fig. 2).

When it was desired to remove one average blade from each plant in a certain row, the lowest green blade was clipped from the first plant, the

second blade from the second plant, the third blade from the third plant, and so on for twelve plants, twelve being the average number of blades on the plants. The process was repeated beginning with the thirteenth plant, from which the lowest blade was removed.

Two blades were removed from each plant by cutting off the first and sixth from the first plant, the second and seventh from the second, the third and eighth from the third, and so on. Another series was treated by removing three blades from each plant, another by removing four, and so on to the last row, which had all blades removed. These treatments were administered at different stages in the plant's development. The appearance of the plants with three, eight, and all blades removed is shown in figure 2.

Other types of mutilation were (a) clipping off the tip half of the blades; (b) removal of one side of the blade by splitting it longitudinally by the side of the midrib; (c) stripping the sides of the blades from the midrib but leaving them attached to the plant at their bases; (d) cutting each side of the blade to the midrib in 4-inch segments and removing alternate 4-inch pieces; (e) breaking the midrib of all blades about 3 inches from the stalk; and (f) cutting the blades crosswise to the midrib on both sides about 3 inches from the plant (fig. 3).

The corn used in the tests conducted during the period of experimentation, 1925–1928, was a uniform strain of open-pollinated Reid Yellow Dent. Alternate rows were treated, leaving untreated rows as checks. In 1929 a hybrid corn known as $F_1$365, developed by J. R. Holbert of the U. S. Department of Agriculture, was used. Only hills containing two plants of apparently equal size and stage of development were chosen. The plant occupying the north or west position in the hill was treated by removing the desired number of blades with hand shears. The other plant was left untreated to serve as a check. At harvest time each ear was husked, individually tagged, and stored in a room at 100°–110° F. for curing. When the ears reached a uniform moisture content, they were weighed, measured, and shelled. Also the grain was weighed and the test weight per bushel determined.

In a part of these experiments, an attempt was made to prevent excessive loss of water from the plants through wounds by administering the treatments during cloudy weather or late in the afternoon. This precaution was not taken in all cases, and it is probably of little consequence, for cutting down the transpiration surface occasioned by blade removal would likely lower the rate of water loss from plants much more than the wounds would increase it. WYLIE (**17**) has reported that injured leaves develop protective barriers promptly, thus checking evaporation and preventing fungal invasion.

Experimental results

Influence of stage of development on injury

Corn seedlings may be treated roughly with little or no serious injury. On June 15, 1929, a rain of 0.32 inch was followed by a hailstorm of short duration. Hailstones the size of marbles fell with such force that they dented the soil in the corn field 0.5 inch in depth. Some of them struck the corn plants, tearing the blades severely. Others hit the plants low on the stalk and snapped them off. The plants at that time were about

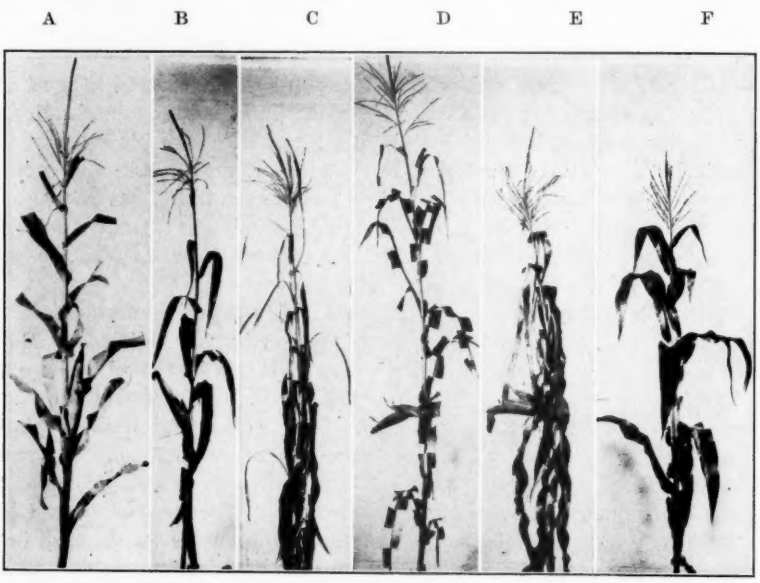

Fig. 3. Types of blade injury used in experiments to determine influence of leaf mutilation upon grain yield: *A*, outer half of each blade removed; *B*, one side of each blade removed; *C*, sides of blades stripped from midrib; *D*, alternate 4-inch segments of blades on each side of midrib removed; *E*, midribs broken about 3 inches from their bases; *F*, blades cut to midrib on both sides about 3 inches from their bases.

5 inches tall. Within two days following the injury, new shoots were plainly visible. These plants grew rapidly, although they were somewhat undersized throughout the season and ripened late.

In order to secure data on recovery following such injury, plants were cut off level with the soil on June 22 and July 2. The average height of the plants at the time of treatment, the number that recovered, and the percentage reduction in yield of grain are presented in table I.

From the results it appears that young corn plants rapidly grow beyond the stage in which they have ability to produce new shoots when the tops are removed. The one plant that survived the treatment on July 2 was

TABLE I

EFFECT OF CUTTING OFF SMALL CORN PLANTS FLUSH WITH SURFACE OF SOIL AT TWO DIFFERENT STAGES OF DEVELOPMENT

DATE OF TREATMENT	AVERAGE HEIGHT TO TIP OF LONGEST BLADE	NUMBER OF PLANTS IN TEST	NUMBER OF PLANTS THAT RECOVERED	NUMBER OF PLANTS THAT PRODUCED GRAIN	REDUCTION IN YIELD OF SHELLED GRAIN COMPARED WITH UNTREATED CHECK
	in.				%
6/22	12.0	29	22	15	87.7
7/2	25.6	23	1	0	100.0

the smallest plant of the twenty-three in the test. It was only 19 inches tall when cut off. All the plants that were 20 inches tall at the time of treatment failed to produce new shoots.

WEIGERT (**16**) observed that young corn plants were much more sensitive to injury from hail than were older ones, for they are more readily

TABLE II

REDUCTION IN YIELD OF GRAIN ASSOCIATED WITH REMOVAL OF ALL BLADES FROM CORN PLANTS AT DIFFERENT STAGES OF DEVELOPMENT

STAGE	REDUCTION OF GRAIN YIELD IN			
	1926	1928	1929	AVERAGE
	%	%	%	%
Vegetative (plants 4 feet tall)	.....	.....	89.34	89.34
Tassel emerging	.....	.....	99.69	99.69
Ear shoot emerging	.....	.....	100.00	100.00
Fresh silk	95.50	.....	98.61	97.06
Early blister	.....	85.76	.....	85.76
Blister	72.94	77.00	76.55	75.50
Early milk	54.55	59.40	.....	56.98
Milk	.....	.....	50.18	50.18
Late milk	26.49	.....	.....	26.49
Glaze	16.95	.....	.....	16.95
Early dent	.....	+1.15	10.14	4.50
Dent	.....	+1.15	4.94	1.90

broken off. In later stages of development the injury consists mainly in the slitting of leaves, and only the extraordinarily severe strokes of hail break the stalks.

In order to test the ability of corn plants to recover from severe hail injury, all blades were removed at twelve different stages of growth, beginning when the plants were 4 feet tall and ending when the grain was well dented. The results, covering a 3-year period, expressed in terms of percentage reduction in yield of shelled corn, are presented in table II.

The greatest yield reduction occurred when all blades were removed during the period between tassel emergence and the fresh silk stage. Treatment earlier than this was not so detrimental to yield because some new leaves are unrolled with the elongation of the tassel-bearing stem. The data obtained suggest that the curve showing the percentage reduction in yield due to removal of all blades at successive stages of growth would start at zero for treatment soon after plumage emergence, and would mount gradually, reaching approximately 90 per cent. at the late vegetative stage, as indicated by the dotted line in figure 4. According to data secured, the

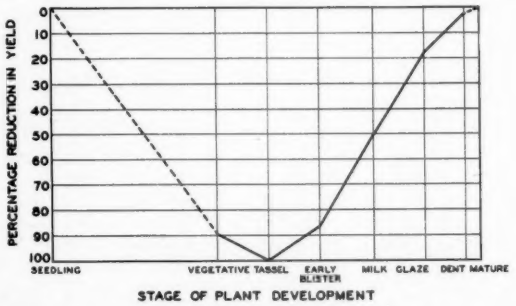

FIG. 4. Reduction in yield of corn following removal of all blades at different stages of plant and grain development (solid line drawn from data; broken line represents an estimation).

loss of grain reached a maximum of 100 per cent. for treatment at pollination time and dropped gradually as the grain developed, reaching the zero point at complete maturity (fig. 4).

EFFECT OF ARTIFICIAL HAILING AND BLADE REMOVAL ON QUALITY OF GRAIN

The quality of the grain also was modified in these tests by the removal of all blades. Table III contains data on the percentage reduction in test weight of grain associated with the removal of all blades at different stages of growth.

The greatest decrease in test weight occurred when the plants were

TABLE III

REDUCTION IN TEST WEIGHT OF GRAIN PRODUCED BY PLANTS FROM WHICH ALL BLADES WERE REMOVED AT DIFFERENT STAGES OF DEVELOPMENT

STAGE	REDUCTION OF TEST WEIGHT IN		
	1928	1929	AVERAGE
	%	%	%
Vegetative (plants 4 feet tall)	.....	7.8	7.8
Tassel emerging	.....	No grain	.....
Ear shoot emerging	.....	No grain	.....
Fresh silk	.....	17.6	17.6
Early blister	15.0	.....	15.0
Blister	22.8	23.3	23.1
Early milk	16.1	.....	16.1
Late milk	.....	14.5	14.5
Early dent	1.5	4.8	3.2

stripped of their leaves soon after the fertilization period. Blade removal when the plants were in the vegetative stage reduced yields markedly, but

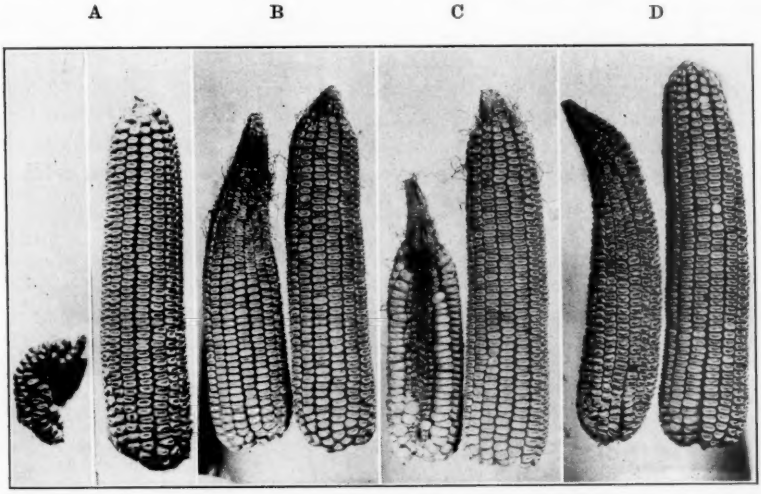

FIG. 5. Different types of adjustment in quantity of grain in response to reduced leaf area. Ears on left were from treated plants, those on right from untreated ones: *A*, cob size greatly reduced following blade removal in vegetative stage; *B*, sacrifice of kernel development at tip in favor of kernels at middle and butt of ear; *C*, four rows of kernels abortive following removal of eight blades at silking time; *D*, general underdevelopment of all kernels as a result of removing all blades when grain was in blister stage.

the degree to which the test weight was lowered did not correspond to the yield reduction, owing to the ability of the less mature plants to adjust the size of ear to the greatly reduced photosynthetic area of the plant. This adjustment was brought about in three different ways. When the blades were removed before or just as the shoot emerged, the size of the cob was greatly reduced and only a comparatively few ovules and silks developed (fig. 5 *A*). When the ovules had been fertilized at the time of treatment, the immature blister-like kernels on the tip of the ear failed to receive further nourishment and therefore were sacrificed in favor of the kernels at the butt (fig. 5 *B*). Under some conditions, the kernels on the lower side of the ear as it hangs on the stalk failed to develop. Reduction of leaf area tended to weaken the shank so that the tip of the ear hung downward, thus inadequately exposing the silks on the under side of the ear to pollen. This resulted in an ear with rows of kernels missing owing to lack of fertilization (fig. 5 *C*). When all blades were removed, further development of the kernels was seriously checked. The ear on the left in figure 5 *D* was produced on a plant that had all blades clipped off when the grain was in the blister stage. Obviously such almost complete cessation of the filling process would have a great effect upon the quality as well as upon the quantity of the grain.

The bruising action of hailstones markedly affected quality of grain. In an experiment to determine the extent of injury from bruising the ears, corn plants were beaten with a whip made of a bundle of baling wire. This implement tore the blades, broke many midribs, and battered the partially developed ears. The data obtained from treating plants in this way at different stages of their development are presented in table IV.

TABLE IV

INFLUENCE OF ARTIFICIAL HAILING WITH A WIRE WHIP UPON PERCENTAGE INCREASE OF ROTTED EARS AND PERCENTAGE DECREASE IN BOTH TEST WEIGHT AND YIELD OF SHELLED CORN, 1927

STAGE	INCREASE IN NUMBER OF ROTTED EARS	REDUCTION IN	
		TEST WEIGHT	YIELD OF GRAIN
	%	%	%
Blister to early milk	118.5	7.27	30.55
Late milk	0	3.87	27.70
Glaze	0	1.47	6.18
Early dent	0	0	2.54

The number of rotted ears was more than doubled by the artificial hailing when the grain was between the blister and early milk stages.

There was no increase in percentage of rotted ears for treatments applied in the late milk or later stages. The test weight per bushel and the yield of grain were decreased by treatments administered later in the plant's development. This decrease was due in large measure to the destruction of the leaf area rather than to the bruising effect of the wire whip on the ears.

Influence of leaf area removed on yield

In estimating the reduction in corn yield resulting from blade removal, it is as important to know the influence of the percentage of leaves destroyed as it is to know the effect of the stage of plant development. Data were therefore obtained on this phase of the problem by removing different percentages of the plant's leaves at various stages of growth (table V and fig. 6).

TABLE V

Reduction in yield of corn as a result of removing different percentages of leaf area at various stages of development, 1929

Blades removed	Reduction in yield of shelled corn as a result of removing blades				
	In tassel with shoot just emerging (July 31)	Fresh silks showing (Aug. 9)	Early blister stage (Aug. 16)	Milk stage (Aug. 30)	Early dent stage (Sept. 12)
%	%	%	%	%	%
8.3	*5.66**	9.00	4.93	5.54	+3.52
16.7	5.18	*12.47*	9.26	*12.98*	+4.13
25.0	*26.19*	*12.14*	*12.47*	*13.37*	7.82
33.3	*28.25*	*24.98*	*26.41*	*17.37*	6.55
50.0	*37.34*	*36.28*	*33.77*	*28.45*	*16.97*
66.7	*49.16*	*52.12*	*44.76*	*21.84*	9.51
83.3	.....	*69.99*	*61.15*	*36.86*	*16.83*
100.0	*100.00*	*98.61*	*76.55*	*50.18*	*10.14*

* Italicized data were accompanied by odds of greater than 30:1.

A reduction in yield resulted when as small a leaf area as 8.3 per cent., which represents one average leaf per plant, was removed. The severity of the injury was roughly in proportion to the number of leaves removed. Even though the data are irregular it may be concluded that the stage of plant development, with the exception of the early dent stage, had little influence upon the yield reduction up to and including the removal of 25 per cent. of the leaves. Losses in the tassel, fresh silk, and early blister stages ran closely together up to the removal of 66.7 per cent. of the blades.

The curves of reduction in grain yield show a slight tendency to be

of an exponential type. Probably the efficiency of the remaining leaves is increased by the removal of blades, thus preventing the grain yields from falling off in direct proportion to the amount of leaf area destroyed. On the other hand, a certain rather constant amount of energy is required to maintain the life of the plant. When this is deducted from that supplied by the products of photosynthesis, there is only a limited quantity

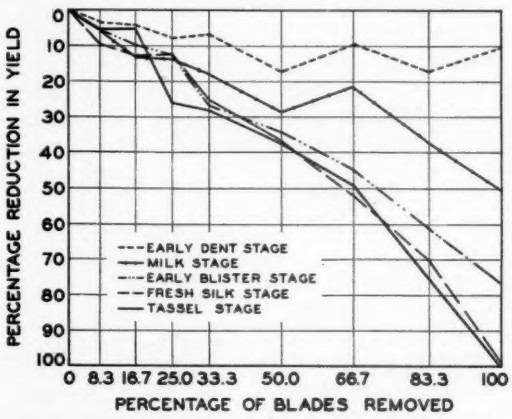

FIG. 6. Percentage reduction of grain yield following removal of different percentages of leaf area of corn plants in different stages of development.

left with which to form grain. With a decreasing supply of carbohydrates available as an increasing number of blades are cut away, the amount remaining to form grain would be disproportionately reduced. Thus the increased efficiency of the remaining blades tending to hold the yield up, and the constant maintenance demand exerting a drain on the elaborated food to the very last, result in a graph of yield reduction that resembles an exponential curve.

INFLUENCE OF REMOVING DIFFERENT PARTS OF BLADES

Since hail riddles the tips of blades badly and usually breaks off pieces of the outer portions, a test was made to determine whether there is any difference in the efficiency of the different parts of the blades. Table VI shows the reduction in yield following the removal of one-half the blade area in three different ways. The outer half of each blade was cut off, one side of the lamina of each blade was torn away, and six average blades were removed. The tests in 1928 included cutting out sections 4 inches long on alternate sides of the midrib. Because of the ease with which the

TABLE VI

COMPARISON OF YIELD REDUCTION ASSOCIATED WITH REMOVAL OF ONE-HALF THE BLADE SURFACE IN DIFFERENT WAYS AT VARIOUS STAGES OF DEVELOPMENT, 1929

STAGE	DATE	REDUCTION IN YIELD					
		OUTER HALF OF EACH BLADE REMOVED		ONE SIDE OF EACH BLADE REMOVED		SIX AVERAGE BLADES REMOVED	
		PERCENTAGE	ODDS	PERCENTAGE	ODDS	PERCENTAGE	ODDS
		%		%		%	
Ear shoots emerging	July 31	23.93	>9999:1	38.26	>9999:1	37.34	>9999:1
Silks fresh	Aug. 9	34.79	>9999:1	38.55	>9999:1	36.44	>9999:1
Early blister	Aug. 16	32.51	>9999:1	34.66	>9999:1	33.77	>9999:1
Milk	Aug. 30	24.11	>9999:1	29.39	>9999:1	28.45	>9999:1
Early dent	Sept. 12	4.15	4:1	8.30	9:1	16.97	1110:1

remaining 4-inch sections were torn off by the wind, this method of removing half the leaf area was not used in 1929.

In the main the yield reduction was practically the same for all three methods of taking off half the leaf area, as shown by the data in table VI. There seemed, however, to be a tendency for the removal of the outer half of each blade to be less injurious than the tearing away of one side of each blade or the cutting away of half the number of leaves. Since the tests conducted the year previous (**5**) with a different variety of corn showed the removal of the tip half of the blades to be slightly more severe than the other methods of removing half the leaf area, it is believed that less than half of the blade area was removed in the 1929 experiment. The blades of the variety designated F_1365, used in 1929, were very wide at the base and it would be easy to make this error. At any rate it seems justifiable to conclude that it is mainly the percentage of blade area destroyed at any one time that influences the yield, rather than the particular portion of the blade that is destroyed.

One of the most common types of injury resulting from hailstorms is the shredding of the blades and the tearing of the laminae of the leaves from the midrib. In order to see how harmful this type of mutilation is, plants were treated by stripping the sides of the blades from the midrib but leaving them attached to the plants at the base (fig. 3 *C*). The data secured from applying this treatment to plants in different stages of development are presented in table VII.

The maximum injury was a little over 25 per cent. Since the reduction in yield for removing all the blades in the tassel-emerging stage was 100 per cent., it appears that the blades torn from the midrib are about

TABLE VII

YIELD REDUCTION WHEN BLADES WERE STRIPPED FROM MIDRIB BUT LEFT ATTACHED TO BASE, 1929

STAGE	DATE	REDUCTION IN YIELD	ODDS
		%	
Vegetative	July 20	15.91	2499:1
Tassel emerging	July 25	25.73	>9999:1
Ear shoots emerging	July 31	25.02	>9999:1
Silks fresh	Aug. 9	22.41	>9999:1
Early blister	Aug. 16	20.47	>9999:1
Milk	Aug. 30	11.97	28:1
Early dent	Sept. 12	2.41	2:1

75 per cent. as efficient as normal ones. HUME and FRANZKE (7) found that splitting the tips of the blades produced an average reduction in yield of 12.2 per cent. when the plants were in the hail-critical stage. Splitting the blades before and after this stage produced smaller reductions in yield.

TABLE VIII

REDUCTION IN YIELD OF GRAIN RESULTING FROM SLIGHT BLADE INJURIES TO CORN PLANTS IN DIFFERENT STAGES OF DEVELOPMENT, 1929

STAGE	DATE	REDUCTION IN YIELD OF GRAIN			
		MIDRIB BROKEN 3 INCHES FROM STALK		BOTH SIDES OF BLADE CUT TO MIDRIB 3 INCHES FROM STALK	
		PERCENTAGE	ODDS	PERCENTAGE	ODDS
		%		%	
Vegetative plants, 4 feet tall	July 20	15.20	475:1	.....	.....
Ear shoots emerging	July 31	5.00	3:1	18.83	434:1
Silks fresh	Aug. 9	11.65	46:1	39.28	>9999:1
Blister	Aug. 16	18.24	3332:1	26.22	>9999:1
Milk	Aug. 30	14.57	434:1	25.76	>9999:1
Early dent	Sept. 12	3.83	3:1	6.95	9:1

EFFECT OF BREAKING MIDRIB AND CUTTING LAMINA

Strong winds often cause the supporting tissues in the midrib to collapse and a hailstorm causes much of this type of injury. In order to test the harmfulness of such breaks in the vascular system, midribs were broken artificially about 3 inches from the base of the blade. As a companion experiment, the laminae were cut to the midrib on each side about 3 inches

from the stalk, making it necessary that all translocative materials pass through the vascular elements of the midrib. The results of applying such treatments at different stages of the plant's development are shown in table VIII.

Cutting the blade to the midrib on each side was associated with a greater reduction in yield than breaking the midrib. The maximum reduction in yield was 39 per cent. for cutting the blades to the midrib and 18 per cent. for breaking the midrib. The results secured in 1928 (5) showed a 23 per cent. reduction as a maximum following the breaking of the midrib and a 20 per cent. maximum for cutting the blade to the midrib. The difference is due to the stronger wind of 1929, which caused the breaking of the midrib of many of the blades that had been cut to this point. These tests show that translocation can take place through the lamina alone or through the midrib alone, without being accompanied with severe reductions in yield. So long as a blade part is attached to the plant, it seemingly possesses the ability to function photosynthetically and to translocate elaborated reserves to the ear.

Blade removal and breaking of ear shanks

A noticeable effect of blade removal was a reduction in the strength of the ear-supporting shank. This was especially apparent when the blades were removed during the earlier stages of the plant's development. A record of the percentage of shanks that were broken in the 1928 tests is presented in table IX.

Since the breaking of shanks is influenced both by the size of the ear and by the strength of supporting tissue, extremely early blade removal would be expected to result in a low percentage of broken shanks because the ears were too small to break them over. When the blades were removed after the ears had developed partially, the weight was sufficient to overtax the strength of the shanks. It is evident that the strengthening fibers of the shanks reach their final development rather late in the growing period, for blade removal when the grain was in the early dent stage resulted in no increase in percentage of broken shanks.

Estimating losses from hail

The problem of estimating losses resulting from hail injury involves a number of considerations. It is difficult to adjust for deteriorations in quality of grain except for the increase in percentage of rotted ears. This can be judged only by husking and examining the ears a considerable time after the storm. It probably is not desirable to attempt to ascertain the loss in quality as expressed by test weight per bushel. Feeding experiments reported by Rusk and Snapp (10) indicate that the dry matter in

TABLE IX

PERCENTAGE OF EAR SHANKS FOUND BROKEN AT HARVEST TIME ON PLANTS FROM WHICH A DIFFERENT NUMBER OF BLADES HAD BEEN REMOVED AT VARIOUS GROWTH STAGES, 1928

NUMBER OF BLADES REMOVED PER PLANT	PERCENTAGE OF SHANKS BROKEN WHEN TREATED ON				AVERAGE OF AUG. 11, 18, AND 28 TREATMENTS
	AUG. 11	AUG. 18	AUG. 28	SEPT. 15	
	%	%	%	%	%
1	51.3	47.2	50.0	61.1	49.5
2	63.2	66.7	70.0	63.9	66.6
3	68.3	71.4	61.5	59.5	67.1
4	78.9	69.4	71.8	58.3	73.4
5	75.7	65.0	61.5	51.4	67.4
6	90.9	76.3	82.9	51.3	83.4
7	82.0	83.3	76.9	64.9	80.7
8	90.0	77.8	80.0	31.2	82.6
9	77.5	83.8	80.0	54.3	80.4
10	81.1	84.2	85.7	.....	83.7
11	92.5	94.9	83.8	.....	90.4
All	91.7	100.0	77.1	64.9	89.9

well preserved, immature corn has practically as high a nutritive value, pound for pound, as the dry matter in sound, mature corn.

Natural hailstorms inflict injury of so many types and degrees of severity that it is next to impossible to duplicate them in experimental tests. The following types of damage may occur in combination on the same plant: (1) loss of blades or portions of blades, (2) shredding of the leaf portions remaining on the stalk, (3) part or all of the midribs broken, and (4) stalks and developing ears more or less severely bruised. Add to this the fact that the severity of natural hail usually varies in different parts of even a small field and the problem of correctly determining the loss appears difficult indeed.

Results of these investigations offer some assistance, however, in that they show the percentage loss in yield that may be expected to follow the removal of a given percentage of the leaf area. Furthermore, they show rather definitely the stage of development at which the corn plant is most susceptible to injury.

The data show that of the factors studied the destruction of blades exerts the greatest influence in the direction of yield reduction. Shredding the laminae from the midrib, even though every blade be so treated, produced a maximum yield reduction of only 25 per cent.

The work of ascertaining the amount of leaf area destroyed may be

done several days or even weeks after the storm, but notes on the stage of the corn development should be carefully taken immediately after the storm has subsided, for data clearly show that the stage of growth influences the severity of the losses as much as does the percentage of the leaf area destroyed.

Summary

1. Experiments covering a period of five years have been conducted with corn in an effort to determine the influence of blade removal, as well as other leaf and plant injuries of the types caused by hail, upon the quality and quantity of grain produced.

2. Although a definite manual of directions for hail insurance adjustment cannot be written at this time, certain fundamental principles are established through these experiments which may well serve as an aid to one's judgment in estimating hail injury.

3. It was found that the critical stage for leaf injury is at the time the ear shoots are just emerging. Leaf removal and injury before or after this stage is progressively less harmful to yields, becoming very slight in the dent stage.

4. The quality of grain as indicated by test weight per bushel was reduced most by leaf removal when the developing grains were in the blister stage. On the other hand, rotting of ears following bruising action of artificial hailing was worst when the treatment was applied during the early milk stage.

5. Tearing the sides of the blades from the midribs but leaving them intact at the base resulted in a yield 75 per cent. higher than complete removal of the blades. This indicates that as long as a leaf blade or part of it is attached to the plant, it is capable of functioning.

6. Reductions in yield at all growth stages correspond roughly with the percentage of leaf area removed, suggesting the possibility of estimating the loss from hailstorms from a determination of the leaf surface destroyed. Suggestions are made for ascertaining losses from hail, using data compiled in these experiments.

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FACTORS IN ELONGATION AND EXPANSION UNDER REDUCED LIGHT INTENSITY

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(WITH FOUR FIGURES)

In seeking to determine the rôle of factor and function in adaptation in nature, the first step has been to deal under control with one factor, and the function directly concerned, by equalizing the others. As an outcome of the results thus secured, it has been possible to modify the method to take into account two variables, such as light and water, in the hope of evaluating their respective rôles. This is particularly desirable in the case of adaptation to shade, in which reduced light intensity has generally been regarded as the controlling agent, with little or no consideration of water relations. Since water serves both as a raw material and as a mechanical force in growth, it would seem on theoretical grounds to play a significant part in such modification. This assumption has been borne out by the behavior of ecads in nature and especially by that of transplants in shade at times and seasons when the water content was deficient. The present account deals with the investigation of the mutual relation of light and water as exhibited by growth and modification in a series of lath-houses, which have been found to afford better control than cloth tents.

Experimental technique

The standard phytometer method was employed, in which are utilized galvanized iron containers 8.5 inches wide and 11 high, provided with removable lids containing a 2-inch hole to accommodate the stem and a watering tube. A layer of coarse gravel an inch thick was placed in the bottom of the can and the weight of the latter then adjusted to a total of 2000 gm. by varying the gravel. Air-dry loam to the amount of 6600 gm. was added, together with 132 gm. of water or 20 per cent. of the dry weight, and the containers left over night to permit even penetration. In filling each can, the glass tube 1.5 cm. across was held against the bottom to prevent clogging and was raised a half centimeter at the first watering to allow ready absorption.

After soaking the sunflower seeds over night, five were placed near the center of the container and allowed to germinate in the greenhouse. By two weeks after planting, the first pair of true leaves were well developed, and all but one of the seedlings in each can were pulled out. In this operation much care was taken to select individuals for uniformity in height and the size of the leaves.

The essential feature of the experimental procedure was to vary the holard under each of several degrees of light intensity, though the installation was improved in certain details through the five series. The first two sets were preliminary in nature and only the two extremes of holard, namely, 35 and 13 per cent., were used. In the remaining series, there were four different water contents and either three or four effective intensities of light. The technique is best illustrated by series V in which 96 phytometers were utilized. These were organized into four batteries of 24 each, to three of which water was added by weight to bring the holard to 35, 26, and 18 per cent. of the dry weight. Since the soil used is saturated at 42 per cent., the values represented respectively 83, 62, and 43 per cent. of saturation. No water was added to the fourth battery, which was allowed to dry out until the water content reached 13 per cent. or 31 per cent. of saturation. A layer of 500 gm. of sand was employed to prevent evaporation but at the same time to permit adequate aeration. Finally, the lid was put on the can, the glass-tube stoppered, and a seal of non-absorbent cotton wrapped about the stem in such manner as to completely close the opening.

In this series the light gradient comprised four intensities, *viz.*, full sunlight, 32, 16, and 8 per cent. of sunlight as afforded by the group of lath-houses. A battery of 24 phytometers was installed in each of the latter, as well as in the sun, where the containers were placed in a wooden box sunken in the soil to prevent overheating of the walls and consequent interference with root functions. As indicated previously, each battery embraced four different holards, represented by six phytometers each. The higher value of 35 per cent. was the maximum for the soil, since an occasional effect of deficient aeration was found in the lower light intensities, while the extreme of 13 per cent. now and then permitted slight wilting under high insolation. A strain of *Helianthus annuus* with simple stems was used in all series, and this was supplemented in the last one by *Clarkia elegans*, a California annual with exceptional adaptability to shade.

Though it was impossible to equalize temperature and humidity in the garden and the lath-houses, the difference in average daily maximum temperature was but 1.5° F. and between average daily minima 4° F. throughout the spring period. There were only 3 degrees' difference in average temperature, this being highest in the lath-house of 16 per cent. intensity and the same in the sun and the lath-house with 8 per cent. The average humidity varied somewhat more, being nearly uniform in the lath-houses but rising several degrees in the sunlight, probably owing to the sea breeze. While the alternation of factor changes is more regular in lath-house than in forest, it is evident that it is of the same nature as that between sun-fleck and shadow below such a canopy.

The water content was kept practically constant by weighing the con-

tainers from time to time and replacing the amount of water lost. Frequent weighings were not necessary during the first few weeks while the plants were small, but as they grew larger, replacement was required more often. During the last week when temperatures were high, some individuals lost more than a liter of water per day in the sun and thus demanded weighing and watering at two-hour intervals to maintain the proper holard. In addition to the water requirement, the rate of growth in stem and leaf was traced week by week, and the wet and dry weights of each plant were ascertained at the close of each series. Measurements of stem height and diameter and of the length and width of each leaf were made each week at the same hour and in the same sequence. Leaf areas were calculated by the formula $L \times W \times 1.3$, which gives the combined area of the two sides (CLEMENTS and GOLDSMITH, "The Phytometer Method," 1924: 35); this method was again verified for the strain of sunflowers employed.

Results from series V

This was not only the most complete of the various series, but it also coincided with the time of the year when the adaptation sequences were at their optimum in the Santa Barbara climate, namely, during April and May. Series IV occupied the period of January to April, and number III that from October to December. The criteria employed were height and diameter of stem, number and area of leaves, wet and dry weights of the whole plant, water requirement and transpiration. For the sake of ready comparison, the data are presented in two sequences, *viz.*, arranged under the four holards in accordance with light intensities, and in the four light values by the percentage of holard.

With respect to the growth of the stem, the results were essentially consistent for diameter, the maximum occurring in 35 per cent. holard and sunlight and falling off rather regularly with reduced light. A slight discrepancy is found in sunshine for 18 per cent. and 13 per cent., owing to the fact that the rate of supply occasionally lagged behind water loss. The response in height was less consistent only to the extent that the maximum alternated between 16 per cent. and 32 per cent. light. On the other hand, the integration of stem growth as represented by height diameter (H/D) yielded a regular rise with decreasing light, only the extreme condition of 13 per cent. holard and 8 per cent. light being out of accord. Leaf area diminished with all but perfect regularity in harmony with light values, dropping very rapidly in the two lowest intensities, while there was no exception to the regular decrease in the number of leaves.

As was anticipated, functions in general and the relatively simple transpiration in particular exhibited the closest correspondence with factor gradients. Water loss was uniformly highest in the sun and fell off sharply

in light of 32 per cent. However, the greatest decrease was between 32 per cent. and 16 per cent., where it was threefold, or even sevenfold in 13 per cent. holard. The loss per cm.^{2} agreed with the total except for the lower three water contents in the 8 per cent. lath-house, a discrepancy probably associated with the small leaf area, crowded stomata, and thinner cuticle. In agreement with transpiration, both wet and dry weights dropped with light intensity, but the dry weight diminished more rapidly, the maximum being respectively 23, 23, 12, and 15 times the minimum in each holard, by contrast with 13, 12, 6, and 8 for the wet. On the other hand, the water

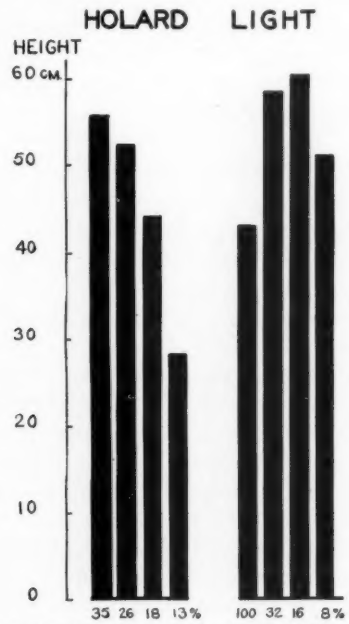

FIG. 1. Average stem heights for holard and light in the three series.

requirement rose steadily with weaker light, with the exception of the 13 per cent. holard, and this is to be explained by the fact that food-making decreased faster than water loss as the energy fell off, as shown by ratios of 23, 23, and 12 in the former, and 15, 11, and 7 in the latter.

Turning to gradients of holard under each of the four light intensities, stems were shorter in response to lower water values, with two exceptions, while diameters lessened in every case. Leaf areas followed the rule with but two insignificant discrepancies, and the number of leaves decreased throughout. Total transpiration is similarly in accord with the holard, as

is likewise the wet weight. Dry weight is slightly less harmonious, lack of aeration becoming perceptible in 35 per cent. holard, while the water requirement is all but entirely consistent.

Results from series III and IV

In addition to *Helianthus,* series IV contained *Clarkia elegans,* a native annual especially tolerant of shade. The holard gradient was the same, but the reduced light intensities were but two, namely, 16 per cent. and 8 per cent. As a consequence, the tallest stems were always found in the 16 per cent. value, while as before the stature was distinctly greater in 8 per cent. than in sunshine. The diameter of stem diminished consistently with the

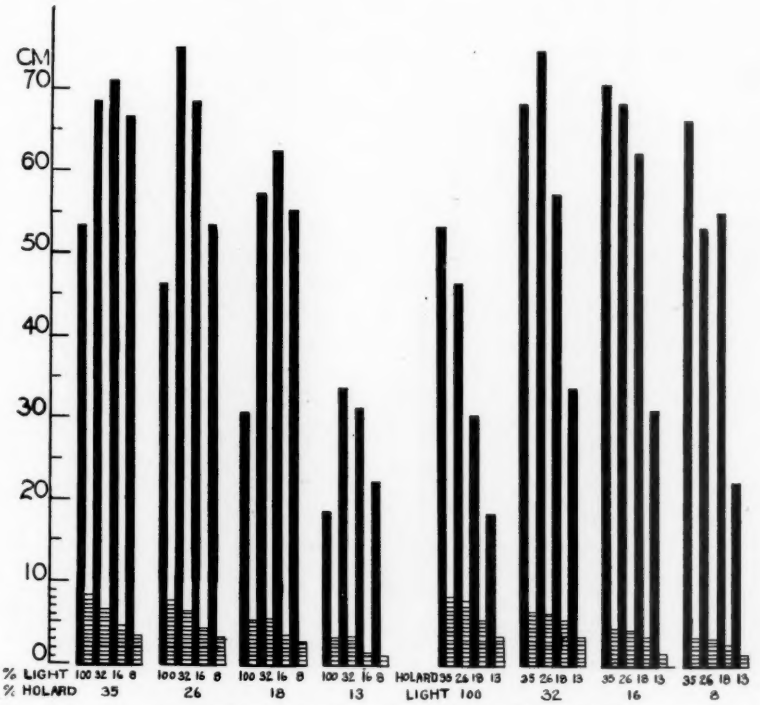

FIG. 2. Stem height (×1) and diameter (×5) in series V.

three intensities, as did also leaf area, number of leaves, wet and dry weights; conversely, height/diameter of stem was enhanced. Total transpiration fell off in entire harmony with light reduction, as did likewise the water loss per cm.,^{2} with the exception of a slight rise in 8 per cent. under

13 per cent. holard. By contrast, the water requirement rose regularly except for a small discrepancy in 8 per cent. light and 26 per cent. water content. The values for the holards within each light intensity were less consistent, the plants in 26 per cent. usually growing better than those in 35 per cent. This discrepancy seems to be explained by the fact that this

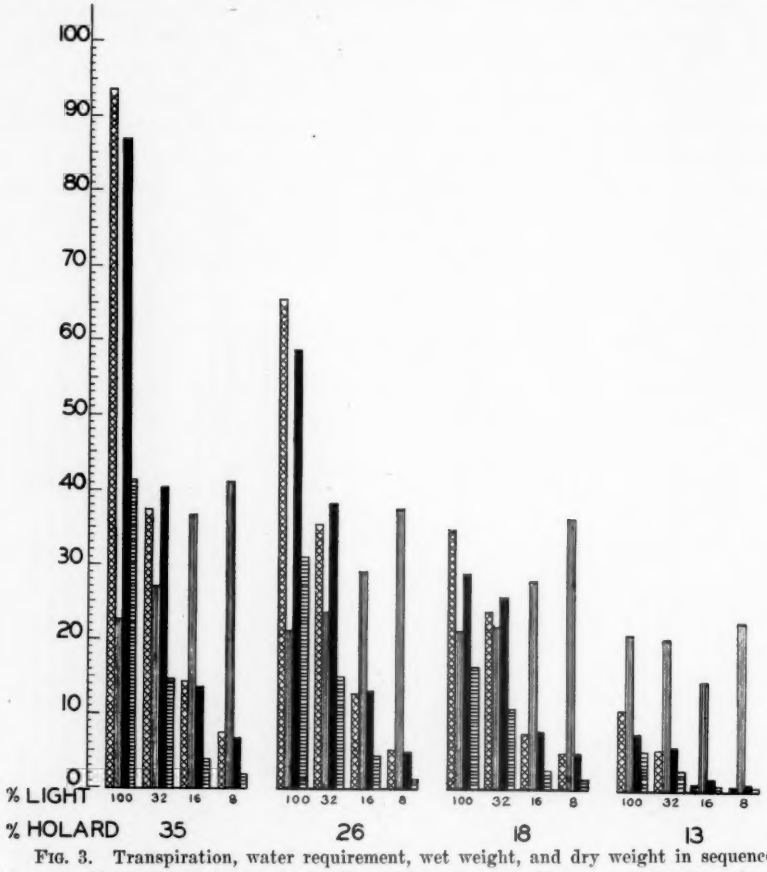

FIG. 3. Transpiration, water requirement, wet weight, and dry weight in sequence for series V, the basing unit representing 100 cc. transpiration per plant, 10 cc./gm. water requirement, 4 gm. wet wt. and 1 gm. dry wt.

series was begun in January when transpiration did not compensate for the high water content and the resulting tendency toward deficient aeration.

The relations of stem and leaf to the factor gradients were distinctly less uniform for *Clarkia*. Wet and dry weights dropped consistently with

both light and holard, as did likewise the total transpiration, but maximum stature and leaf size alternated between sun and 16 per cent. light. Stem diameter was more regular in response, but water requirement was quite erratic. The divergence in behavior as compared with the sunflower is probably to be ascribed to the necessity of transplanting the individuals as seedlings, thus favoring the production of lateral shoots to the disadvantage of the main stem.

Series III represented the original installation that was duplicated in IV, and was grown from October to December of the preceding autumn. The two corresponded almost exactly in the trend of results, except that water requirement in the earlier set was uniformly higher in 16 per cent. light.

For comparison with the response in the lath-houses where the light reduction was produced by a moving shadow, sunflower batteries were placed under light arcs approximately 10 ft. at the base, 6 ft. high and 3 ft. wide. One of these was covered with cheesecloth, the other with paper, yielding respective light values of 62 and 14 per cent., by contrast with full sunshine. For the gradient, the values were the most variable obtained during the entire course of the experiment. Height of stem, leaf area and number, wet weight, and water requirement were not concordant in the four holards, nor with the results in other series. However, diameter gave a positive correlation with decreasing radiant energy, as did dry weight and total transpiration, a single marked exception occurring in 13 per cent. holard in the sun, where wilting disturbed the relation. The values for sunlight and 62 per cent. light were regularly much greater than for 14 per cent., as would be expected, but they were also much closer to each other and alternated more frequently than the difference in intensity would warrant on the basis of a direct relation. Aside from water requirement, the values for the holard sequence in each light intensity were essentially consistent throughout, except for a slight decrease in the 35 per cent. in both degrees of shade, due it would seem, to deficient aeration.

In order to test the rôle of water in combination with another factor, a third subseries of the same four holards was grown under three wind conditions, namely, 0, 5, and 10 miles per hour. Apart from the 5-mile wind under 13 per cent. water content, the results as to growth and water requirement were practically all consistent. Stem height and width gave a negative correlation with increasing velocity, the two being in harmony with each other instead of opposed, as in the case of light. The leaf area and number also fell off as the wind velocity rose, as did wet and dry weight, while the water requirement was augmented. The total transpiration was highly variable, but the water loss per cm.^{2} rose with much regu-

TABLE I

RESULTS FROM SERIES V

FACTOR GRADIENTS		STEM					LEAF AREA PER PLANT		LEAVES	
		HEIGHT		DIAM.		H/D				
HOLARD	LIGHT									
%	%	*cm.*	%	*cm.*	%		*sq. cm.*	%	*No.*	%
35	100	53.3	100	1.66	100	32.1	4556.6	100.0	23.7	100
	32	68.3	128	1.30	78	52.5	3124.5	69.0	18.5	78
	16	70.9	133	0.91	55	72.8	1122.3	25.0	14.3	60
	8	66.3	125	0.68	41	97.5	648.6	14.0	12.5	53
	Ave.	64.7	122	1.14	69	63.7	2458.5	52.0	17.2	73
26	100	46.3	87	1.58	95	23.9	3420.0	75.0	22.2	94
	32	75.0	141	1.25	75	60.0	2641.5	58.0	18.0	76
	16	68.3	128	0.83	50	82.3	1195.5	26.0	13.2	56
	8	53.5	100	0.61	37	87.8	435.8	10.0	11.3	48
	Ave.	60.8	114	1.07	64	63.5	1923.2	42.0	16.2	69
18	100	30.5	57	1.05	63	29.1	1743.3	38.0	17.2	73
	32	57.3	108	1.08	65	53.0	1939.1	43.0	15.2	64
	16	62.5	117	0.71	43	88.0	727.2	16.0	12.3	52
	8	55.3	104	0.55	33	100.5	439.6	10.0	11.0	46
	Ave.	51.4	97	0.85	51	67.9	1212.3	27.0	13.9	59
13	100	18.8	35	0.65	39	28.9	521.5	11.4	12.2	51
	32	33.6	63	0.66	40	51.0	499.6	10.9	10.5	44
	16	31.0	58	0.28	17	111.0	114.2	2.5	8.2	35
	8	22.1	41	0.23	14	96.0	65.2	1.4	6.1	26
	Ave.	26.4	49	0.46	28	71.7	300.1	6.6	9.3	39

FACTOR GRADIENTS		TRANSPIRATION		WEIGHT OF PLANT			WATER REQUIREMENT	
		TOTAL	GM./SQ. CM. MAY 7–14	WET	DRY	RELATIVE WEIGHT		
HOLARD	LIGHT							
%	%	*cc.*	*gm.*	*gm.*	*gm.*	%	*cc./gm.*	%
35	100	9359.0	134.5	347.7	41.23	100.0	227	100.0
	32	3727.0	80.9	162.1	13.70	33.0	272	120.0
	16	1428.0	78.5	54.2	3.88	9.4	368	162.0
	8	741.5	64.2	26.6	1.80	4.4	412	181.0
	Ave.	3563.9	89.5	147.7	15.15	36.7	320	141.0
26	100	6564.3	99.6	234.9	31.02	75.0	211	92.9
	32	3535.5	78.4	153.2	15.00	36.0	236	104.0
	16	1290.0	64.5	52.4	4.43	11.0	291	128.0
	8	508.1	68.5	19.1	1.35	3.3	376	165.6
	Ave.	2974.5	77.8	114.9	12.95	31.3	279	122.6
18	100	3647.0	103.0	115.7	16.35	40.0	212	93.4
	32	2383.6	70.5	102.7	10.95	27.0	218	96.0
	16	748.5	59.0	31.8	2.68	6.5	279	122.9
	8	485.1	61.1	19.5	1.33	3.2	365	160.8
	Ave.	1771.1	80.9	67.4	7.83	19.2	269	118.3
13	100	1057.0	114.7	30.0	5.08	12.3	208	91.6
	32	527.5	69.1	22.8	2.63	6.4	201	88.5
	16	83.8	52.2	5.9	0.58	1.4	145	63.9
	8	79.1	79.7	3.7	0.35	0.9	226	99.0
	Ave.	434.9	78.9	15.6	2.16	5.2	195	85.7

TABLE I—(*Continued*)

RESULTS FROM SERIES V

FACTOR GRADIENTS		STEM					LEAF AREA PER PLANT		LEAVES	
LIGHT	HOLARD	HEIGHT		DIAM.		H/D				
%	%	*cm.*	%	*cm.*	%		*sq. cm.*	%	*No.*	%
100	35	53.3	100	1.66	100	32.1	4556.6	100.0	23.7	100
	26	46.3	87	1.58	95	29.3	3420.0	75.0	22.2	94
	18	30.5	57	1.05	63	29.1	1743.3	38.0	17.2	73
	13	18.8	35	0.65	39	28.9	521.5	11.4	12.2	51
	Ave.	37.2	70	1.24	74	29.9	2560.3	56.1	18.8	80
32	35	68.3	128	1.30	78	52.5	3124.5	69.0	18.5	78
	26	75.0	141	1.25	75	60.0	2641.5	58.0	18.0	76
	18	57.3	108	1.08	65	53.0	1939.1	43.0	15.2	64
	13	33.6	63	0.66	40	51.0	499.6	10.9	10.5	44
	Ave.	58.5	110	1.08	65	54.1	2051.2	45.2	15.6	66
16	35	70.9	133	0.91	55	72.8	1122.3	25.0	14.3	60
	26	68.3	128	0.83	50	82.3	1195.5	26.0	13.2	56
	18	62.5	117	0.71	43	88.0	727.2	16.0	12.3	52
	13	31.0	58	0.28	17	111.0	114.2	2.5	8.2	35
	Ave.	58.2	109	0.68	41	88.5	789.8	17.4	12.0	51
8	35	66.3	125	0.68	41	97.5	648.6	14.0	12.5	53
	26	53.5	100	0.61	37	87.8	435.8	10.0	11.3	48
	18	55.3	104	0.55	33	100.5	439.6	10.0	11.0	46
	13	22.1	41	0.23	14	96.0	65.2	1.4	6.1	26
	Ave.	49.3	93	0.52	31	95.5	397.6	8.9	10.2	43

FACTOR GRADIENTS		TRANSPIRATION		WEIGHT OF PLANT			WATER REQUIREMENT	
LIGHT	HOLARD	TOTAL	GM./SQ. CM. MAY 7–14	WET	DRY	RELATIVE WEIGHT		
%	%	*cc.*	*gm.*	*gm.*	*gm.*	%	*cc./gm.*	%
100	35	9359.0	134.5	347.7	41.23	100.0	227	100.0
	26	6564.3	99.6	234.9	31.02	75.0	211	92.9
	18	3467.0	103.0	115.7	16.35	40.0	212	93.4
	13	1057.0	114.7	30.0	5.08	12.3	208	91.6
	Ave.	5111.8	113.0	182.1	23.42	56.8	215	94.5
32	35	3727.0	80.9	162.1	13.70	33.0	272	120.0
	26	3535.5	78.4	153.2	15.00	36.0	236	104.0
	18	2383.6	70.5	102.7	10.95	27.0	218	96.0
	13	527.5	69.1	22.8	2.63	64.0	208	88.5
	Ave.	2544.4	74.7	110.2	10.57	40.0	234	102.1
16	35	1428.0	78.5	54.2	3.88	9.4	368	162.0
	26	1290.0	64.5	52.4	4.43	11.0	291	128.0
	18	748.5	59.0	31.8	2.68	6.5	279	122.9
	13	83.8	52.2	5.9	0.58	1.4	145	63.9
	Ave.	887.6	63.6	46.0	2.89	7.1	271	119.2
8	35	741.5	64.2	26.6	1.80	4.4	412	181.0
	26	508.1	68.5	19.1	1.35	3.3	376	165.6
	18	485.1	61.1	19.5	1.35	3.2	365	160.8
	13	79.1	79.7	3.7	0.35	0.9	226	99.0
	Ave.	453.5	68.4	17.2	1.21	2.9	345	151.5

larity in the higher water contents, the relative values being 2, 3, 5 for 35 per cent. and 1, 2, 3 for 18 per cent.

With respect to the holard gradient under the several velocities, stem height and diameter diminished with reduced water values, and this was equally true of leaf area and number, as likewise of wet and dry weights. The same rule held for water requirement with a single exception, and applied consistently to the total water loss but less regularly to transpiration per unit of leaf area.

TABLE II

COMPARISON OF RESULTS FROM THE THREE SERIES

	HOLARD-LIGHT			LIGHT-HOLARD		
	V	IV	III	V	IV	III
Stem						
height	m16–32	m16	m16	−r	m26	−r
diam.	−r	−c	−r	−c	−r	−r
ht./diam.	+r	+c	+r	d	−d	−r
Leaf						
area	−r	−c	−c	−r	m26	−c
number	−c	−c	−c	−c	m26	−r
Weight						
wet	−c	−c	−c	−c	m26	−c
dry	−c	−c	−c	−r	m26	−r
Transpiration						
total	−c	−c	−c	−c	m26	−r
per cm.²	−r	−r	−d	−d	−r	−d
Water require.	+r	+r	m16	−r	d	d

In the above table, positive correlation with the decreasing gradient of light or holard is shown by the minus, negative by the plus sign: *c*, denotes consistent, *r*, regular, and *d*, discrepant. Where the maximum is out of the sequence, it is indicated by *m* with a figure for the condition. The agreement between the three series is close in the case of light, especially in view of the fact that the sun's altitude was respectively that of spring, winter, and fall. The accord is distinctly less for holard, by reason of the divergence of the fourth series for the most part, but also, it would seem, because of the unavoidable variation of holard in the interval between two waterings.

When the results in stem height are averaged for the three series, they yield the following percentages in the order of descending holard, *viz.*, 100, 93, 77, and 50, while for decreasing light the values are 100, 136, 140, and 118. For stem diameter, the respective figures in the same sequence are

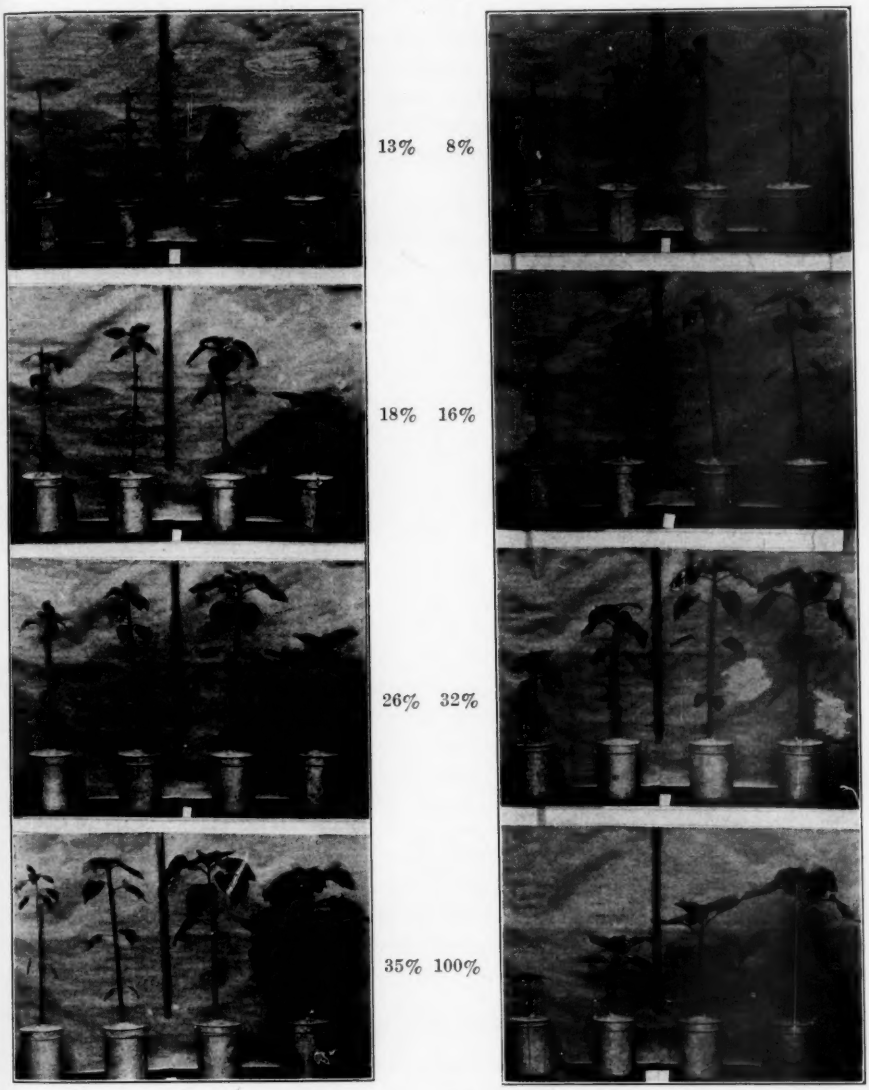

FIG. 4. A representative set from each battery of phytometers in series V, showing the growth under the various conditions.

100, 99, 78, and 58, and 100, 82, 61, and 49. Dry weight finds its maximum of 121 per cent. in 26 per cent. holard, falling then to 65 and 28, but for the light gradient it falls regularly and quite consistently from 100 to 45, 16 and 7 per cent. The grand averages for area and number of leaves drop with almost complete regularity for each factor, but while the five largest leaves for 35 per cent. holard are found in the sun, for all other water contents they occur in 32 per cent. light.

TABLE III

EXPANSION OF LARGEST LEAVES, SHOWN IN CM.^{2}

HOLARD	LIGHT INTENSITY			
%	% 100	% 32	% 16	% 8
35	553.8	475.0	204.0	141.2
26	378.8	416.2	205.8	98.2
18	303.4	352.0	144.0	101.2
13	127.2	144.6	29.4	17.3

There is evidently a negative correlation between the expansion of the leaf and its thickness, but there are several difficulties in the way of the ready determination of the latter. These reside chiefly in the leaf itself and especially in the complication introduced by the veins. Variations in turgor are a further source of error, as is also the actual technique of measuring thickness on leaves in position. If allowance be made for shrinkage, the simplest method is to make measurements on microtome sections from representative areas on which to base a range or average. The histological material obtained from the various series is yet to be worked up in connection with similar adaptations in native transplants, but a preliminary study of leaf thickness has been made for series V. The averages for each holard gradient in the four light intensities are in fair correspondence with the latter, the thickness falling rapidly from sun to 32 and 16 per cent. and then slightly to 8 per cent., *viz.*, 0.2923, 0.2462, 0.2016, and 0.2002.

Relative rôles of water and light

In all the series, it is obvious that water plays a considerable part in the elongation of stems and the expansion of leaves. This is naturally to be expected, chiefly on the basis of its mechanical action in turgor, but to some extent also because of its use as raw material, and this is supported by the

responses in both native and control cultures. More difficult of answer, however, is the question as to the respective importance of water and light in growth under reduced light intensities. Probably the frequent statement that plants stretch up in the shade in order to secure more light is intended to be more or less figurative, but the response is so graphic as to seem to lend support to this explanation. The major objective of the present investigation is to discover whether the relation to each of the factors permits quantitative expression.

TABLE IV

MAXIMUM DIFFERENCES BETWEEN EXTREMES

LIGHT EFFECTS			
HOLARD	STEM HEIGHT	DRY WEIGHT	LARGEST LEAVES
%	*cm.*	*gm.*	*cm.*^{2}
35	17.6	39.43	334
26	28.7	28.67	318
18	32.0	15.02	251
13	14.8	4.63	127
Average	23.3	22.19	257
LIGHT		HOLARD EFFECTS	
%	*cm.*	*gm.*	*cm.*^{2}
100	34.5	35.15	426
32	41.4	11.07	330
16	39.9	3.30	175
8	44.2	1.45	124
Average	40.0	12.74	264

The averages of the maximum differences for light and for water responses deviated little from each other in the case of the largest leaves, and this was true likewise of leaf area and stem diameter. However, both stem height and dry weight exhibited striking divergences. The maximum difference in stature produced by light within the various holards ranged from 14.8 to 32 cm., with an average of 23.3 cm., while for the holard gradient within each light set the respective values were 34.5 and 44.2 cm., with an average of 40 cm. Similarly, in the table for series V, the differences between the extreme averages for light intensities, namely, 37.2 and 58.5, is 21.3 cm., while for the four holards the corresponding figures are 64.7 and 26.4, with a difference of 38.3 cm. Hence, the excess of stature

in terms of response to water over that to light is 16.7 cm. in one case and 17 in the other. For all three series the difference between the greatest and least average height for the holard was 27.7 cm.; for light, it was 17.3 cm. On the basis of the average height for all series and conditions, the difference, 10.3 cm., amounted to 21 per cent. in favor of water.

With respect to the dry matter produced, the situation was reversed. The differences between extremes ranged from 4.63 to 39.43 gm. with an average of 22.19 gm. for light, to respective values of 1.45 to 35.15 gm. and an average of 12.74 gm. for water. For the three series, the difference between the highest and lowest averages was 12.33 gm. for water and 21.44 gm. for light. The excess to be ascribed to light is 8.9 gm., which approximates closely the average dry weight for all series and conditions, namely, 9.1 gm., and thus indicates that the rôle of light in producing construction material is twice as great as that of water. This relation is reflected in the production of photosynthate as measured by the picric acid method. From 8 per cent. light to full sunshine the amount rose from 0.384 to 1.408 mg. per cm.^{2}, a gain of 1.028 mg., while for the increase from 13 per cent. to 35 per cent. holard, the rise in sugars was but 0.45 mg. This was somewhat less than half as much, but the departure from the value derived from the dry weights is not serious.

From the foregoing, the conclusion seems warranted that variations in holard exert a minor effect upon the amount of material for the construction of stems while light plays the paramount rôle. On the other hand, the mechanical action of water is increased as the resistance offered by materials drops in proportion to photosynthesis. This would seem to find expression chiefly in the stem where the ratio between dry matter and sap-content is low by contrast with the leaf where the ratio is high. This is further indicated by the small differences in the averages for largest leaves and leaf areas (table IV). However, the results obtained during the past fifteen years in growing nearly a hundred species in lath-houses, length-of-day tents, in water and nutrient sequences, as well as a much larger number in transplant gardens, demonstrate that there may be considerable differences in behavior between species and genera as well as between life forms.

The major rôle assumed by the holard in the elongation of shoots serves to explain several apparent contradictions in the behavior of transplants, as well as of species subjected to factor gradients. At the outset it was expected that the transfer of alpine dwarfs from the summit of Pike's Peak to the plains at 6000 ft. or to the sea coast at Santa Barbara would produce an increase of stature. This did not happen in most cases and it was soon realized that this must be due to increased transpiration in the presence of a holard often tending toward a deficit. In short, the striking change of

climate had little or no effect upon elongation except during wet seasons, and to modify the dwarf habit effectively it became necessary to maintain an adequate water content. As to the prevalence of dwarfing in the alpine tundra itself, it had been earlier discovered that this was characteristic of climax and subclimax communities. Wherever disturbance reduced competition for water, as in the so-called "gopher gardens," or the supply was adequate as in wet meadows, the stature increased from two to fourfold, to equal that at much lower altitudes.

The controlling influence of water has also become more and more evident in natural shade gardens located in the spruce climax at 8000 ft. At the time of installation it was assumed that survival would be greater and modification more rapid than in transplanting into the sun, but the reverse often proved true, especially as to survival. This was found to be due to high interception of the frequent but light showers by the canopy and even more to the thick layer of needles and duff that decreased penetration to the root level. It was thought to cure this by sprinkling, but it finally became necessary to remove much of the duff to assure a proper water content. This accomplished, the shade gardens became the most prolific in striking modifications and pointed the way to the greater control and success achieved by means of lath-houses at the three altitudes.

Summary

Phytometer batteries of sunflowers have been employed in combinations of four degrees of holard with four of light to determine the respective rôles of these two factors in the apparent response to shade. The results obtained indicate that water assumes the major rôle in stem elongation, and light the larger part in the production of dry matter, the two necessarily cooperating in the adaptation characteristic of shady habitats.

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THE COHESION THEORY OF TRANSPIRATION

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(WITH TWO FIGURES)

The present explanation of the mechanism of water transport in plants (**11,** p. 9; **12,** p. 231) includes, briefly, the pulling of continuous columns of water from the soil by means of evaporation from the leaves, the columns remaining intact under the stress of great height and rapid evaporation because of the tensile strength of the water (**6, 16, 20**).

WALTER (**23,** p. 67), after a discussion of experiments on water transport, says, "Damit scheint die Theorie der Wasserleitung, die auf der Kontinuität der Wasserfäden von der Spitze bis zur Basis der Pflanzen beruht und deshalb als Kohäsionstheorie bezeichnet wird, endgultig gefestigt zu sein, wenn auch im Einzelnen immer noch Veränderungen eintreten können. . . ."

WOODHOUSE (**24**) reports experiments interpreted as indicating a need for modification of the tensile column theory. The experiments are: first, an inability, using *Pittosporum undulatum* and *Ricinus communis,* to draw mercury to a height greater than 49 cm., and, second, a greater speed of evaporation from a branch of *Ricinus* on a day with moderate conditions of evaporation than on a day of intense evaporation.

In the first case the inability to draw mercury to a height greater than 49 cm. can be explained as due to failure to clean and prepare the apparatus sufficiently and to remove air from the plant stem. BOEHM (**4, 5**) found that out of hundreds of experiments to demonstrate the rise of mercury above atmospheric pressure only a few were successful. With more refined methods THUT (**18**) and URSPRUNG (**21**) demonstrated the tensile strength of a column of water using living material. THUT (**17**) and OTIS (**13**) have described methods for its demonstration in a physical system. The experiments are sufficiently reliable to use in classroom exercises.

In the second case the difference in rate of evaporation of branches of castor bean on the warm and on the cool day can be ascribed to a probable difference in leaf area since this factor apparently was not evaluated. No arguments are presented to support the claim that the development of greater tension on a day of less evaporation makes the tensile column theory inadequate.

In addition WOODHOUSE interprets experiments with a 14-meter glass U-tube as throwing doubt on the tensile column theory. "The top of one arm of the U-tube was connected by a stopcock to a vacuum system. It was hoped that the velocity of the water, descending in one arm of the tube and

rising in the other, combined with its cohesive properties, would carry it to an elevation greater than the barometric column. Unlike ASKENASY (**3**) [**1**] and THUT (**102**) [**17**] under the conditions of this experiment no tensile column was obtained. The water entered the vacuum chamber in spurts, and numerous bubbles formed in the column."

In connection with this experiment it is important to consider the condition at the top of any column of water in which its cohesive properties are to be demonstrated. According to RENNER (**14**): "Die Höhe der als unzerreissbar angenommen Wassersäule, die in einer beliebig weiten Röhre an einer porösen Membran aufgehängt werden kann, hängt von der Porenweite dieser Membran ab. Die Wassersäule kann genau so hoch werden, wie das Wasser in einer Kapillarröhre steigen würde, deren Weite auf ihrer ganzen Länge gleich der Weite der gröbsten Poren der Membran ist."

In the experiment of WOODHOUSE the diameter at the top of the glass tube in contact with the gas in the vacuum chamber was 1.75 mm. The top of the water column was not attached in any way and could not be supported by its tensile strength. The height to which water rises in such a tube is determined by the difference in gas pressure between the base and top of the column and the force of surface tension at the top. The surface tension in a tube of the size used will support a column of water to a height of 18 mm. as calculated from the formula $h = 2\sigma/rdg$. The experiment demonstrates a water barometer. The bubbles appearing in the water when the vacuum was applied were very likely due to expansion of air bubbles present in the joints sealed with de Khotinsky cement.

WOODHOUSE reports a modification of the experiment in which a thread was run through the entire tube. In this case a continuous column was obtained unbroken by air bubbles. Presumably this column was supported by additional air pressure at the base since the author states that no tensile column was obtained yet shows a height of 14 meters in the figure (p. 188, **24**). The rate of flow (in a tube containing water and with a thread from top to bottom) when air pressure at the base (*i.e.*, vacuum at the top plus additional pressure at the base) is released and the tube allowed to empty is used as an argument for a combined tensile strength-sorption hypothesis. The curve obtained deserves attention. Extension across the abscissa indicates that with negative heights the velocity of descent continues to increase in contradiction to accepted laws of hydrostatics and free energy. It is difficult from the description of the experiment to explain this curve in any way. It is possible that the de Khotinsky cement extending into the lumen of the tube at certain points may have caused the results.

In modifying the tensile column theory, WOODHOUSE calls sorption into consideration, a factor whose importance in water transport in plants has been evaluated by SHULL (**15**). WOODHOUSE extends the sphere of influence

of the imbibitional or adsorptive forces of the wall material to the center of the smaller xylem vessels, suggesting a limiting distance of 5 microns for the action of this force. BODE (**3**) is quoted, " 'Dagegen war das Eindringen von Luft in Gefässe mit einem Durchmesser unter 10 μ nie zu beobachten.' " The heading of this portion of BODE's paper is "2. Die Wasserfäden in mechanisch verletzten Gefässen." In an earlier portion of his paper under the heading "1. Im intakten Gefäss" BODE states, "Ergebnis: Die Pflanze war vollkommen schlaff, und dennoch blieben sämtliche Wasserfäden intakt!!" This was the result after working on *Elatostemma sessile* which has vessels as large as 50 μ in diameter.

Aside from the fact that the hypothesis proposed by WOODHOUSE does not account for active conduction in vessels exceeding 10 μ in diameter, sorption, if acting to the distance and with the force postulated, is an *impediment rather than an aid* to water movement since imbibition forces sufficient to hold water against the pull of gravity would act as effectively along the tube against the pull of transpiration. In his hypothesis the tension of the water is suggested as the motive force while imbibition on the wall is one of support only. But, accordingly, to cause water movement the tensile force must overcome that of imbibition as well as that of gravity before motion can occur. If the imbibition forces are effective to the distance assumed by WOODHOUSE they should be sufficient to prevent the pushing of water through the 10 μ xylem vessels of a short piece of branch carefully cut at both ends to which a force of 1 atmosphere is applied. DIXON (**6**) obtained flow of water through wood of *Taxus baccata* using a head of water equal to the length of the branch. The diameters of the tracheids of this plant were not stated but presumably they were not much larger than 10 μ, while the diameter of the pores in the walls of the tracheids did not exceed 0.3 μ.

Experiments

To establish a complete water column supported by its adsorptive and cohesive forces the apparatus shown in figure 1 was set up. The tubes AB and CD were formed by melting with a gas-oxygen flame two Pyrex tubes 60 by 1.5 cm. and gradually drawing them out in a stair well into a tube of varying size, about 1.5 to 6 mm. inside diameter. The final length was 13.5 meters. After both tubes were drawn they were sealed together at the top so that a continuous glass siphon was formed. In order to insure sorption of the water on the glass, potassium bichromate in sulphuric acid was forced from D to A by means of a tire pump and valve at F. The flask used was a 250-cc. round-bottomed Pyrex flask. The rubber stoppers were wired in place to hold the pressure which was estimated by means of the manometer G. To obtain fairly rapid passage of the solution it was necessary to employ pressures of as much as five atmospheres. When the tube had been filled

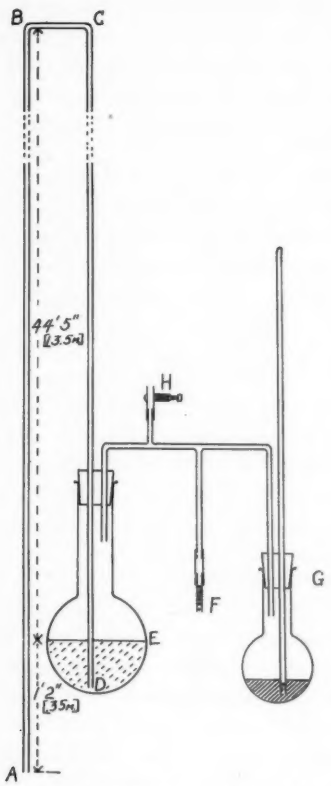

FIG. 1. Apparatus to demonstrate cohesion of water column.

with the cleaning solution alcohol was poured down the outside from the top and ignited when it reached the bottom. This heating of the solution in the tube insured thorough cleaning.

1. COHESION OF A COLUMN OF MOVING AIR-FREE WATER

The cleaning solution in the tube was followed by boiling water and this by boiled water which was allowed to cool in the tubes. The pressure in the flask was released by unscrewing the clamp H. The water column did not break and the 14-inch difference in level between A and E caused water to drip from A. After about 30 cc. of water left in the flask were emptied the ascending meniscus moved up the shorter arm DC with increasing speed as the difference in water level increased.

2. Cohesion of a column of moving air-containing water

The experiment was performed as before except that unboiled distilled water was substituted for the boiled water. This distilled water had been standing in a 5-gallon bottle open to the air. In this experiment the water column remained intact when pressure was released at the base and siphoned for six minutes, emptying 20 cc. of water at the base of the lower arm. The column then broke ten feet from the top of the tube DC, probably due to the conduction above 32 feet of some impurity in the distilled water. The flow before breaking was sufficient to show that the cohesive force of a moving solution of air in water is strong enough to support the water column to a height of 44 feet.

3. Force of water sorption in an agar gel

Tube AB was disconnected from CD at the top of the column and one end of a short piece of glass tubing 3 cm. in diameter was sealed on to the open top end. The junction of the small and the 3-cm. tubing was then narrowed to an area of about 3 sq. mm. The top of the 3-cm. tubing was left open to the atmosphere. Boiled water was forced up the tube after cleaning with cleaning solution and allowed to cool. The pressure at the base was maintained so that the water column was supported but did not change in level. Two per cent. agar solution was prepared and filtered while hot through a fritted glass filter. A piece of absorbent cotton was then dipped in the agar, taking care that no air bubbles were included in the interstices of the cotton which was then placed in the top of the water column and forced down into the constriction. The excess water in the top portion of the tube was replaced by agar and the cotton and agar allowed to set. The column remained intact when the pressure at the base was released.

It was originally hoped that evaporation from the surface of the agar would result in drawing water up the tube. To this end a waxed paper cylinder was tied over the open end of the tube containing the agar and connected to a beaker of sulphuric acid. The agar gradually decreased in volume and shrank away from the sides of the tube until at the end of 78 hours the shrinking allowed air to slip past the cotton-agar plug and the column broke. Only a part of the water leaving the agar gel was absorbed by the sulphuric acid, since 2 cc. of water were collected in a 10-cc. graduated cylinder placed at the bottom open end of the tube to measure the volume change. That this was not a volume change due to temperature difference was evidenced by the fact that the increase in water at the bottom was gradual during the time that the column was intact. The tension (less than $\frac{1}{2}$ atmosphere) at this height was sufficient to remove some of the water from the gel. The imbibition forces in the gel are insufficient to hold all the water against this pull. If the experiment had continued until equilibrium

had been established and no more water withdrawn, presumably evaporation from the surface of the agar would have resulted in pulling water up the tube.

Under such conditions of equilibrium the water bound in the gel by a force of about 0.5 atmosphere could be determined by drying the agar and weighing the residual water. Although at completion of the experiment equilibrium had not been reached, a considerable part of the water in the gel was withdrawn as is indicated by the figures (table I) for the 78-hour period.

TABLE I

Agar in the original gel	2	per cent.
Weight of agar, water, and cotton after 78 hours	6.40	gm.
" " " and cotton	0.46	gm.
" " cotton after boiling in water and drying	0.28	gm.
" " agar	0.18	gm.
" " water	5.94	gm.
Percentage agar after 78 hours of 0.5 atm. tension	2.94	per cent.
Percentage of water removed from agar during tension	32.7	per cent.

Of the water lost from the agar, 2 cc. were collected in the graduated cylinder at the bottom. The rest was absorbed in the sulphuric acid at the top of the column.

4. Cohesion of a stationary column of air-containing water

It was believed that difficulties in demonstrating the cohesive force of air-containing water were not so much due to dissolved air as to the presence of small particulate impurities with adsorbed air which might provide a nucleus for collection of dissolved air (**6**). The importance of such nuclei can be qualitatively observed in a dirty beaker in which tap water is heated as compared with a beaker cleaned in very hot cleaning solution. In the first case bubbles gradually collect on the walls of the beaker as the temperature rises, whereas in the second case few bubbles are to be observed. The influence of dirt on the boiling point was noted by Gay-Lussac [after Ursprung (**19**)]. Observations similar in principle indicating the importance of particles in allowing condensation of water vapor from the air are known from meteorological observations as well as from experiments in photographing electron paths.

Instead of boiling the water the results of experiment 3 suggested the possibility of filtering out all particulate impurities by means of the agar. A tube similar to that of experiment 3 was prepared except that it was drawn quickly from Pyrex tubing 3 cm. in diameter, forming a capillary 13.9 m. long, of diameter 0.6 mm. in the center to 1.4 mm. in diameter at a

distance 1 m. from the end. It was cleaned, filled with boiled, cooled water, and plugged with a cotton-agar plug. After the gel had set, the pressure at the base was released with no resultant break in the water column. Eight cc. of 0.25 per cent. acid fuchsin solution were poured on top of the agar. The top was closed to evaporation by tying over it a waxed paper bag. The layer of agar and cotton was about 2.5 cm. in thickness, tapering from a diameter of 3 cm. at the top to one of 1.5 mm. where the capillary joined the large tube. Below the constriction in the water-filled portion of the tube the diameter was 6 mm. In 8 days the acid fuchsin solution on top of the agar had been sucked through and an equivalent amount of water had collected in the flask at the bottom of the capillary. The red color of the dye could be observed extending from the agar down the capillary to a distance of 1 meter. Unless by some means the air in the dye solution was removed during the passage through the agar the water at the top of the column now contained dissolved air. It did not break.

To obtain a greater suction tension the container at the base of the capillary was gradually evacuated to determine at what tensile stress the column would break. When the manometer indicated 35.3 cm. Hg the sudden increase of water in the flask indicated that the column had broken. The break occurred at the junction of the agar-cotton gel with the water column. The barometric pressure was 75.3 cm. Hg. Calculation shows that the break occurred under a tension of 0.9 atmospheres. Thus a column of air-containing water is supported by its cohesive force to a height of at least 9 meters (19 meters when open to the atmosphere).

5. Effect of tension on viscosity of water in a capillary tube

In connection with the speeds of water movement found in plants (8), it is of interest to determine whether viscosity in a water column decreases with tension. Two capillary tubes were drawn out to a distance of about 35 feet in the same way as in experiment 4. These were sealed together at the top and at the base were sealed into flasks as indicated in figure 2. With the apparatus as shown it was possible to apply pressure or suction at will to either or both of the flasks at the bases of the capillary tubes and to count very accurately the flow of water by means of the drops from the capillary in the lower flask. After cleaning and filling with boiled distilled water the flasks and tubes were allowed to cool and then to siphon for two hours. By this time a temperature equilibrium with the surroundings had been reached.

Measurements were taken: (1) when the water in the flasks at the base was subjected to atmospheric pressure; (2) with an additional pressure of 40 cm. Hg applied to both of the flasks; (3) at atmospheric pressure as in

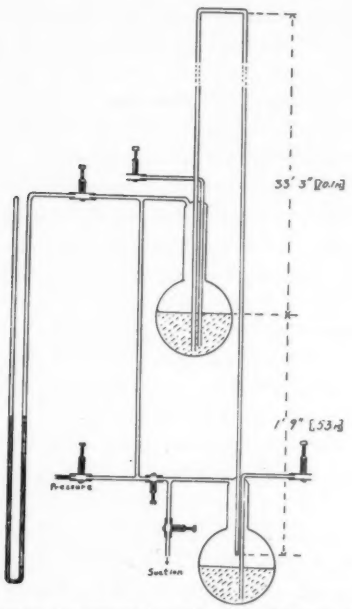

FIG. 2. Apparatus for measuring viscosity of water under tension.

1; and (4) with the flasks at the base both evacuated to a pressure of 27 cm. Hg. The results are given in table II.

Changes in viscosity with this tension change in the water are not sufficient to be detected with the method of measurement used. An experiment was started to determine the above rates with the use of a stopwatch but was discontinued when one of the flasks burst under the pressure used in filling the tube.

It is apparent that the method of measuring the siphoned water depends upon its surface tension as it drops from the lower capillary. In case the

TABLE II

MEASUREMENTS OF THE VISCOSITY OF WATER AT DIFFERENT TENSIONS

PRESSURE	TIME	NO. DROPS	RATE
76 cm. Hg	3 min. 55 sec.	20	11.75 drops/sec.
116 cm. Hg	3 min. 54 sec.	20	11.7 drops/sec.
76 cm. Hg	1 min. 57 sec.	10	11.7 drops/sec.
76 cm. Hg	1 min. 57 sec.	10	11.7 drops/sec.
27 cm. Hg	1 min. 56 sec.	10	11.6 drops/sec.

surface tension of the water changes in exactly the same way as the viscosity when subjected to varying tension or pressure the above experiment is invalidated. It would be necessary in such case to substitute some other means of measuring the water.

Discussion

To obtain consistent results in experiments in which the forces of adsorption and cohesion are concerned it is important to work with materials and surfaces that are clean and homogeneous. The variations in the experimental results obtained by various workers who have attempted to measure the cohesive forces of water are probably due to the variations in the degree to which they succeeded in cleaning the apparatus with which they worked.

In connection with impurities in the water it is important to distinguish between the state of different kinds of impurities. Gases, solids, and liquids dissolved in water are present in dispersed molecular or few molecular units. Any tendency of these impurities to lessen the cohesive force of the water is spread evenly throughout the mass of the water and not concentrated at a surface. (When concentration of the solute occurs at the surface as in soap solutions there is a very great effect on the surface tension and perhaps a corresponding effect on the cohesive force.) Some substances in solution may increase the cohesive force of the water.

The cell wall absorbs water with great force, insuring thorough wetting of the surface with which the water is in contact. That imbibing colloids such as the cell wall need not act to prevent movements of water through them when subjected to tension is indicated by the passage of the water through the agar gel. This may explain in part the way in which the transpiration stream travels from cell to cell in the water-conducting systems of some plants.

That a gel when subjected to a suction tension may shrink while still maintaining its organization and structure intact is indicated by the behavior of the agar gel. The relatively low suction tension force used in extracting water from the gel shows that the force of water sorption is low for a considerable proportion of the water held in the gel. Since the agar gel is a colloid the solid matter composing it may be assumed to be dispersed in units of about 5–200 mμ in diameter. It seems not unreasonable to assume that the diameter of the water-filled spaces in the solid phase does not exceed 200 mμ. The spaces are not visible microscopically. If the water spaces in the gel are of this size it must be concluded that the forces of water sorption of any magnitude are confined to a very short distance in the colloid since with a relatively low suction tension a considerable amount of this water can be removed. It seems probable that in the water-conducting cells of the plant great forces of water sorption act for very short dis-

tances and insure a wall which will act efficiently in excluding air bubbles and in providing an easily wetted surface, but they do not entirely impede the movement of water in the liquid phase within the colloid, much less in the lumen of conducting cells.

The results obtained in experiment 3 suggest that by using tensions of water a quantitative measure of the amounts of water held in certain colloids can be determined as well as the forces with which they are held. It may be that some modification of the scheme could be used to determine the amounts and forces concerned in the "binding" of water in non-living and perhaps in living systems.

The variations in trunk diameter of trees (**10, 7**) seem quite reasonable if we suppose that the tension of the water during periods of rapid transpiration draws a certain small amount of water out of the colloidal material of which the cell wall is composed. When the tension is released the imbibitional forces of the cell wall cause an absorption of water into the interstices of the colloid composing the wall and an increase in the size of the cell. It is of interest to speculate as to what effect the shrinkage of the cellulose in the wall might have on the ease with which water columns under heavy tension might be broken. It would seem that the shrinkage should decrease the ease with which air might penetrate into the vessels. Another effect of wall shrinkage may be to increase the rigidity of cells subjected to a tension so that leaf cells under normal tensions do not wrinkle and collapse but remain in approximately the same shape as when distended by turgor.

URSPRUNG and BLUM (**22**) have measured suction tensions in various parts of the plant and shown that the forces found are in accord with those needed in a cohesion theory of sap transport. To the writer this theory provides a coherent picture of the hydraulics of transpiration, a picture supported by many diverse experiments all indicating the simplicity and clarity of the central idea.

If we accept the physical forces operating at the surface of the leaf as the moving factors in supplying water to the plant, the constant wilting coefficient of a given soil for many kinds of plants finds a possible explanation. Evaporation from the mesophyll cells of the leaves draws ultimately upon the water in the soil. Since the walls of the evaporating cells of plants are composed of substances physically similar, celluloses, it is reasonable to conclude that they will have a similar maximum force which they can exert upon the water in the soil. Wilting occurs when the force with which water is held in the soil exceeds the force which the evaporating surface of the leaf can exert. Use of an instrument absorbing water through physical forces (**9**) has given a fairly constant value for the wilting points of various soils, a value which, if expressed in absolute amounts, is quite variable.

Summary

1. The results of certain experiments interpreted as throwing doubt on the tensile column theory of the ascent of sap can be explained as due to inadequate technique.

2. The tension-sorption hypothesis to explain ascent of sap during active transpiration is experimentally unsupported.

3. Reports of cohesive forces in air-free and air-containing water are substantiated.

4. A method is indicated by which bound water may be determined.

5. Within the limits of error of 1 per cent. no change in viscosity with a tension change of 1 atmosphere was found although the method of measuring the water may have hidden such changes.

6. A mechanism is suggested by which size changes without form changes may occur in both hard and soft parts of plants.

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FREEZING PHENOMENA IN CRESOAP EMULSIONS OF PETROLEUM OILS[1]

PAUL A. YOUNG

(WITH PLATE II AND TWO FIGURES)

Introduction

Studies of freezing emulsions were included in the experiments conducted during 1928 to 1933, to determine whether freezing of oil sprays on apple buds increased the injurious effects of the oils. The protoplasm in the buds is a hydrosol resembling oil-in-water emulsions, so these liquids may behave similarly in freezing. Hence the phenomena of freezing in oil-in-water emulsions were studied to try to reveal information helpful in elucidating the effects of freezing in protoplasm.

Materials and methods

Emulsions of oils 4, 16, 24, and the other oils described in table I, were frozen at $-3°$ to $-30°$ C. in many experiments. Some samples of oils 16 and 24 were saturated with the oil red O stain before emulsification. The emulsions were studied while they were frozen, and also while they were freezing and melting, to observe how they differed from unfrozen emulsions.

Distilled water, and 0.25 per cent. of amorphous carbon suspended in distilled water, were frozen separately for comparison with frozen emulsions. Gas was removed from eight emulsions and from boiled distilled water by evacuating them with a Cenco Rotovac pump before these liquids were frozen. Many emulsions were frozen and melted two or three successive times to determine whether this increased the amounts of oils liberated.

Oils 4, 16, 24, and 36 were made miscible by dissolving 5.5 per cent. of potassium-fish-oil soap and 4.5 per cent. of cresols in them in making the stocks of cresoap-miscible oils (**1**). A high-speed electric stirrer and warm tap water were used in emulsifying the miscible oils and in diluting the emulsions.

Emulsions were frozen in 170 test tubes having dimensions of 100–300 by 15–25 mm., and on 50 microscope slides with and without cover glasses. Thick layers of emulsions were made on some slides by supporting the cover glasses with fragments of broken cover glasses. Melting of emulsions was studied by holding hot needles near them.

[1] Paper no. 36 of the Journal Series of the Montana Agricultural Experiment Station. Contribution from the Department of Botany and Bacteriology.

TABLE I

PETROLEUM OILS USED IN THE STUDIES OF FREEZING EMULSIONS

OIL NUMBER	NAME OF OIL*	PERCENTAGE SULPHONATABLE	VISCOSITY†	PERCENTAGE OIL IN EMULSIONS
		%	*sec.*	%
4	Standard (Cal.) Mineral Seal 4, 13607R	10	50	4
16	Shell 3	6	55	1, 2, 4, 8
24	Sonneborn Amalie 10680P	0	67	2, 10
29	Standard (Ind.) Dendrol Miscible	30	83	4, 8
30	Standard (Ind.) Verdol Emulsion	4	83	5.5
36	Shell E515	14	75	4, 8

* The writer wishes to thank the Standard Oil Co. (Cal.), Standard Oil Co. (Ind.), Shell Oil Co., and L. Sonneborn Sons, Inc. (N. Y.), for supplying the oils and the information about the viscosities and sulphonatable residues of the oils.

† Viscosity in seconds determined with the Saybolt Universal viscosimeter.

Experimental results

EMULSIONS FROZEN ON MICROSCOPE SLIDES

The emulsions frozen on microscope slides had the macroscopic appearance of feathery frost on window glass (fig. 1).

Magnifications of 50 to 600 times revealed the following sequence of phenomena in the cresoap emulsions of 2 and 4 per cent. oils 4, 16, and 24 while they were frozen, and while they were freezing and melting:

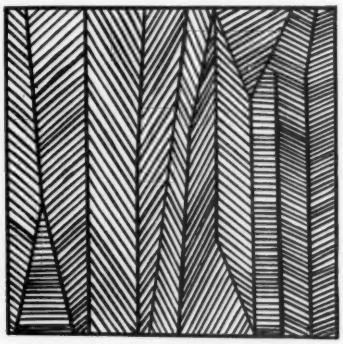

FIG. 1. Diagrammatic drawing of a cresoap emulsion of 2 per cent. oil frozen on a microscope slide. Black lines between transparent ice crystals represent main and branch channels of oil, gas, and aqueous solution of cresols and soaps. ×10.

a) Long needles of ice rapidly invaded fields of emulsions, crowding the oil globules into narrowing channels between the ice crystals, and pressing many oil globules together until they coalesced to form elongate masses of oil (fig. 2 *A*). Oil globules and masses occurred in straight rows as long as 1.7 mm. Gas was evolved while ice crystals formed in freezing emulsions (fig. 2 *D*).

b) Spaces containing gas or aqueous solutions appeared as blue lines nearly 0.2 μ wide between adjacent ice crystals (fig. 2 *B*). Light from these lines was refracted by the microscope lens, making the lines look blue. Horizontal lamellae of ice apparently formed first on the bottoms of the emulsion layers on slides, probably because the slides were laid on ice or stone presumably colder than the surrounding air. Impurities were forced into the liquid above these original crystals so that the crystals touched and formed the blue lines.

c) New horizontal lamellae of ice formed on this bottom layer until all available water was crystallized. Many of these superimposed crystals were separated by wide channels of gas, and by channels of aqueous solution of gas, cresols, and soaps surrounding masses of oil (fig. 2 *C, D, E*). These channels were compressed between the ice crystals, and lay above many of the blue lines.

d) As frozen emulsions melted, widening channels of clear water replaced the blue lines. The channels of oil, gas, and aqueous solution widened as ice crystals melted, and the elongate bodies of oil and gas suddenly became circular in outlines. Many gas bubbles 1 to 20 μ in diameter dissolved and disappeared in the warming water.

e) Oil globules moved rapidly and dispersed in the widening channels of water between melting crystals of ice. Many of the large masses of oils partly or entirely re-emulsified in the water liberated from the ice. Freezing and melting apparently increased slightly the predominating sizes of the oil globules on many slides.

The channels of oil, aqueous solution, and gas between ice crystals were straighter and less numerous in the frozen emulsions of 2 per cent. oil than in those of 4 per cent. oil. Many masses of oil were not inclosed in the channels between ice crystals (fig. 2 *D*). Some of these masses appeared to lie between the ice and the cover glass. Ice frozen from the evacuated emulsions had fewer gas channels and bubbles than ice frozen from the unevacuated emulsions.

Aqueous solution, oil, and gas in the channels between the ice crystals had similar appearances, but dark-field illumination left only the oil prominently visible. Oils also were made distinguishable when freezing precipitated the oil red O in them, and when melting of ice crystals released irregular masses of oils.

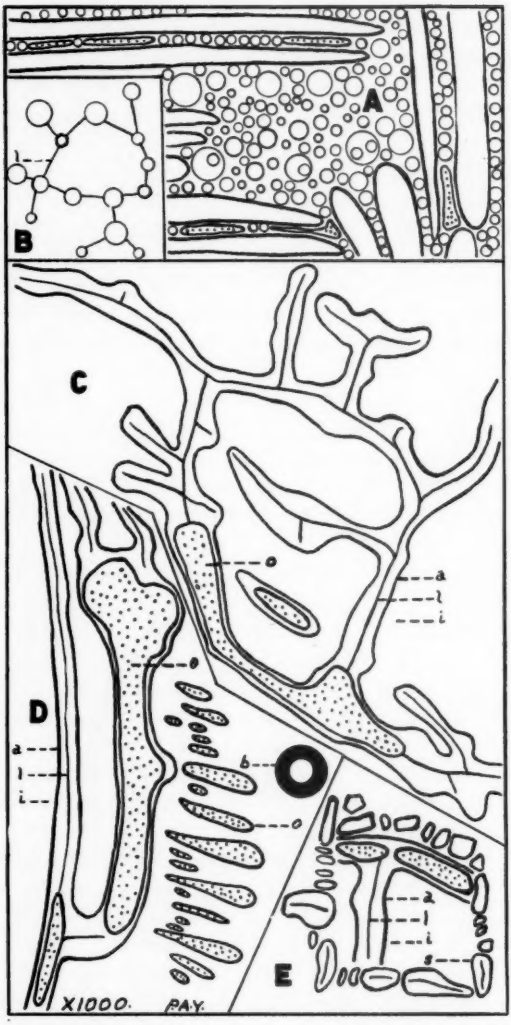

FIG. 2. Diagrammatic drawing showing common arrangements of ice, oil, gas, and aqueous solution of gas, soaps, and cresols in cresoap emulsions of 2 and 4 per cent. oils frozen on microscope slides. Stippled areas represent irregular masses of oil deformed by ice crystals. In parts *B* to *E*, the lines (*l*) represent the blue lines of gas and liquid between nearly touching ice crystals.

A. Needle-shaped crystals of ice invading a field of oil globules (circles). Six irregular masses of oil are compressed between ice needles.

Similar freezing phenomena developed in the emulsions of oils 4, 16, and 24, and oils 16 and 24 saturated with oil red O. These phenomena also were similar in thick and thin layers of emulsions on slides with and without cover-glasses.

Water and carbon suspension frozen on microscope slides

Unlike the ice in frozen emulsions, the ice that formed in boiled evacuated distilled water showed only a few wide channels of gas, and probably a little liquid water saturated with gas. Most of the blue lines separating crystals were alone, but wide channels of gas lay above a few of them. Gas bubbles rose from some of the blue lines (spaces) between the ice crystals when this ice melted and the water became warm. Frozen tap water was similar, but had many more of the wide channels of gas above the blue lines.

The frozen suspension of amorphous carbon exhibited the carbon particles aggregated with gas in the channels; the phenomena otherwise resembled those in frozen tap water. Melting the ice on two of the slides left polygons of carbon particles apparently adhering to the slides.

Emulsions frozen in test tubes

The emulsions freezing in test tubes displayed the following sequence of phenomena:

a) Layers of transparent ice 1 to 7 mm. thick formed near the glass, concentrating the oil globules, gas bubbles, and aqueous solution of gas, cresols, and soaps near the centers of the tubes (pl. II *F*). Parts of the outer transparent ice commonly contained long, parallel, or radial pockets of gas.

b) Needles (pl. II *A*), lamellae (pl. II *D, E*), and polyhedrons (pl. II *A, C*) of ice formed in the emulsions, concentrating the oil globules,

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B. Spherical globules of oil (circles) above the corners of ice-polyhedrons.

C, D. Frozen cresoap emulsion of 4 per cent. oil 4 showing ramifying channels (*a*) of gas and unfrozen aqueous solution of cresols and soaps, lying over blue lines (*l*) between ice crystals (*i*). Higher lamellae of ice inclose and determine the forms of the channels. Irregular masses of oil (*o*) are inside and outside the channels. The thick-margined circle (*b*) represents a large uncompressed sphere of gas.

E. Frozen cresoap emulsion of 4 per cent. oil 16 saturated with oil red O showing masses of unfrozen aqueous solution of cresols and soaps (*s*) arranged as discontinuous channels.

C, D, E. Emulsions frozen at $-6°$ C. The channels (*a*) had dimensions of 10–645 by 5–30 μ. The main channels were 2.5–225 μ apart and the branch channels were 8 to 75 μ apart. The irregular oil masses (*o*) had dimensions of 35–120 by 7–21 μ, and the spherical oil globules were 0.5–20 μ (mostly 1–8 μ) in diameter. Before the cresoap emulsions were frozen, oil globules 0.1–8 μ in diameter predominated in them.

gas bubbles, and aqueous solution of gas, cresols, and soaps between these compound ice crystals (**4**). The lamellae of ice resembled those frozen in dairy cream and milk. Lamellae, needles, and polyhedrons of ice were not conspicuous in part of the emulsions. Ice columns looked milky and opaque during this stage because milky emulsions remained between the ice crystals.

c) Further freezing enlarged the ice crystals by removing water from the channels until the milky-ice columns became translucent and non-milky, apparently because the lamellae and polyhedrons of ice held masses of oils, gas, and concentrated aqueous solution of gas, soaps, and cresols in such narrow layers and channels between them (pl. II *C, D, E*). During this stage the ice columns expanded and pressed against the curved bottoms of the test tubes. This forced the ice columns upward, and usually prevented breaking of the glass tubes. However, rapid freezing of emulsions at $-20°$ to $-30°$ C. broke many test tubes.

d) One to four horizontal cracks developed in most of the expanding columns of freezing emulsions (pl. II *A, B, E, F*). The formation and shapes of these cracks were not altered by making constrictions or bends in the test tubes, or by inclining the tubes at 45° angles instead of holding them in the usual vertical positions. The cracks were milky at first, and developed in non-milky columns of ice. Then ice needles and lamellae entered the cracks and they also became non-milky ice. Red-stained oil indicated that the cracks contained less oil than the other parts of the ice columns. Many ice columns were slipped from their test tubes and were broken easily only at the cracks.

The formation of milky emulsion was the first macroscopic evidence of melting of frozen emulsions (pl. II *D*).

Microscopical observations on the melting of the frozen emulsions showed that the oil globules 0.1 to 20 μ in diameter usually became redispersed in water liberated from the ice. Also many of the larger masses of oils partly re-emulsified in the water. Convection currents in water melting from ice probably facilitated emulsification.

Freezing and melting, especially when repeated, liberated 60 to 90 per cent. of the oils in the emulsions of 1 and 2 per cent. oil; 5 to 10 (sometimes 50) per cent. of the oils in the emulsions of 4 per cent. oil; but apparently liberated no oils from the emulsions of 8 per cent. oil. The emulsions were not agitated artificially while freezing and melting.

Frozen columns of emulsions of 4 per cent. oils 4 and 16 were allowed to remain undisturbed in their test tubes while melting and during a week after melting. The liquids in many of these tubes showed prominent layers bearing different concentrations of oils in the following order from top to bottom: (a) undiluted oil, (b) cream, (c) concentrated emulsion, (d) dilute milky emulsion, and (e) transparent emulsion.

No differences were seen in the freezing phenomena in the emulsions of oils 4, 16, 24, 36, 16 saturated with oil red O, and 24 saturated with oil red O, except that the red-stained oils made pink milky emulsions and red ice.

Freezing an emulsion of 67 per cent. oil 16, emulsified by casein and ammonia, produced many large lamellae of ice like those shown in pl. II *E*. This ammonia-casein-oil emulsion apparently was unaltered by the freezing and melting.

The frozen suspension of 0.25 per cent. amorphous carbon in test tubes showed the carbon particles and gas bubbles to be concentrated in long narrow pockets in the centers of the ice columns. Irregularly horizontal cracks developed in the ice columns. Melting left nearly all of the carbon particles in the bottoms of the test tubes.

Emulsions frozen on apple buds

Emulsions of 4, 6, 8, and 16 per cent. oils froze on apple buds in experiments conducted during 1928 to 1931. There was no evidence that the freezing increased the injurious effects of the oils. The oils emulsified by calcium caseinate usually are liberated quickly without freezing, and most of 4, 6, and 8 per cent. oils emulsified by cresoap re-emulsify in melting, so that freezing is not expected to make oil emulsions more injurious.

Discussion

The following concepts help to explain the freezing phenomena in cresoap emulsions of petroleum oils, and the related phenomena in freezing protoplasm.

In freezing, liquid water excludes other molecules and ions, and forms pure ice when the attractive forces of crystallization between water molecules exceed the forces which prevent freezing. These attractive forces predominate and crystallize pure uncompressed water when enough heat is lost to decrease sufficiently the thermal motions of molecules, and when the latent heat of fusion is liberated. While the thermal motions in solutions decrease below 0° C., the attractive forces between water molecules evidently increase until they overcome the attractions between water and other molecules and ions, and overcome pressures which hinder crystal expansion. Undercooling, pressure, and the existing concentration of non-aqueous molecules, ions, and colloidal particles adsorbing water determine the temperature at which water starts to crystallize below 0° C. This describes phenomena that occur when freezing is relatively slow.

In this way freezing emulsions drove gas from solution and concentrated it with oil and aqueous solution of cresols and soaps in channels and

cavities between compound crystals of ice. Most of the emulsions were undercooled before freezing. Also, pressures strong enough to move and break the ice columns, and break some of the test tubes, developed in the freezing emulsions. Large masses of oil, formed by aggregation of small oil globules, were common in channels in the frozen emulsions. Many small oil globules remained suspended in concentrated aqueous solution of gas, soaps, and cresols between ice crystals. Much oil probably dissolved in such solutions (**3**).

Strong interfacial membranes occur on oil globules containing cresoap. When such oil globules are pressed together, polar radicals on the oriented interfacial soaps probably hold films of water between the oil globules many of which only apparently instead of really coalesce. Compacted, distorted oil globules abound in emulsions of more than 75 per cent. oil (**3**). This helps to explain why melting cresoap emulsions displayed redispersion of small oil globules and partial or nearly complete re-emulsification of large oil masses. Cresoap emulsions of 4, 8, and 10 per cent. oil are more stable than those of 1 and 2 per cent. oil, and this stability characterizes these emulsions during freezing and melting as well as during exposure to constant temperatures.

The cresols and soaps are soluble in water, so presumably they diffuse from globules of cresoap-miscible oils into the water in the emulsions. Minute bubbles of gas also are dispersed in emulsions.

SWINGLE and SNAPP (**2**) determined freezing points of $-0.1°$ to $-1.4°$ C. for different emulsions of $\frac{2}{3}$ oil in water. Apparently the ordinary emulsions cannot be undercooled below $-8.3°$ C. before freezing begins. The freezing points were governed by the emulsifiers.

The observations and interpretations of freezing phenomena in oil emulsions add support to the following concepts in literature about the effects of freezing on protoplasm: (a) Freezing may reduce the water phase below the limit of tolerance in protoplasm unprepared for such dehydration. Naturally increasing the concentrations of solutes and hydrophilic colloids in protoplasm retards and decreases the release of water in freezing and thus lowers freezing points of protoplasm. (b) Ice crystals rupture and crush cell structures.

Further information about freezing will be valuable in studying refrigeration and winter hardiness of plants.

Summary

1. Microscopic freezing phenomena in cresoap emulsions of 2 and 4 per cent. oils on microscope slides were: (a) Needles and lamellae of ice invaded fields of oil globules, arranged them in rows, and caused many of them to coalesce. (b) Compound ice crystals enlarged until adjacent ones were

separated only by narrow spaces which appeared as blue lines. (c) Above these blue lines, other layers of ice inclosed irregular masses of oils, gas, and aqueous solution of gas, cresols, and soaps in irregular channels.

2. As thermal motions of molecules decrease below 0° C., the attractive forces causing crystallization increase until they exclude impurities and aggregate the hydrogen and oxygen of water into crystals of pure ice. This explains theoretically why the dissolved oxygen, nitrogen, cresols, and soaps were excluded from crystallizing water. Removal of the water concentrated these impurities, and crystal expansion inclosed them between the ice crystals.

3. Emulsions frozen on slides had the macroscopic appearance of feathery frost on glass windows.

4. Macroscopic freezing phenomena in cresoap emulsions of 2, 4, and 8 per cent. oil in test tubes were: (a) Clear ice with or without gas bubbles formed near the glass and concentrated the emulsions near the centers of the tubes. (b) Needles, lamellae, and polyhedrons of compound ice crystals formed, inclosing the oils, gas bubbles, and aqueous solutions of emulsifiers in the interstices. (c) The freezing columns of emulsions changed from milky to non-milky appearance, expanded, and formed usually horizontal milky cracks that later froze and also became non-milky.

5. Freezing and melting liberated 60 to 90 per cent. of the oils from emulsions of 1 and 2 per cent. oil; 5 to 10 (sometimes 50) per cent. of the oils in emulsions of 4 per cent. oil; but apparently liberated no oils from emulsions of 8 per cent. oil. Oil globules and large parts of the irregular masses of oil re-emulsified when the ice melted.

6. Freezing of emulsions on apple buds apparently did not increase oil injuries.

The writer wishes to thank Dr. W. M. MARTIN and Dr. R. A. MELAVEN of the Chemistry Department, and Professors A. J. M. JOHNSON and J. A. KIEFER of the Physics Department of Montana State College for their valuable suggestions.

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EXPLANATION OF PLATE II

A. Needles and polyhedrons of ice with milky emulsion between them shown in a freezing cresoap emulsion of 4 per cent. oil 16 saturated with oil red O. Horizontal crack formed near the base of the ice column. ×2.

B. Horizontal crack formed in ice frozen from the emulsion described in *A*. ×2.

C. Polyhedrons and lamellae of ice with concentrated layers of a cresoap emulsion of 8 per cent. oil 16 between them. ×2.

D. Oblique lamellae of ice with concentrated emulsion layers of 8 per cent. oil 29 between them. The emulsion began to melt during photographing and released some milky emulsion. ×1.

E. Vertical lamellae of ice with layers of a cresoap emulsion of 8 per cent. oil 16 between them. Horizontal cracks are shown. ×2.

F. Translucent, non-milky ice surrounding a central column of milky ice, with one horizontal crack in a frozen emulsion of 5.5 per cent. oil 30. Oil globules 2 to 12 μ in diameter predominated in the emulsion before freezing. The melting occurred in the outer layer of non-oily ice, so masses of water with circular and elliptical outlines appeared near the glass; this contrasts with the milky emulsion shown in *D*. ×1.

YOUNG: CRESOAP EMULSIONS

PRODUCTION OF VITAMINS BY A PURE CULTURE OF *CHLOROCOCCUM* GROWN IN DARKNESS ON A SYNTHETIC MEDIUM

M. F. Gunderson and C. E. Skinner

(with two figures)

Introduction

Although considerable information has accumulated with regard to the occurrence of vitamins in plants and animals, little pure culture work has been done for the purpose of studying the physiology of their production by plants.

The data reported here are concerned with a species of *Chlorococcum*,[1] a green alga, isolated from soil. The organism can be grown on a purely inorganic substrate with natural or artificial light as the source of energy, or it can be cultivated, as in these experiments, in complete darkness with a carbohydrate as the source of energy. By using algal cells grown in this manner, it is possible to separate the factor of light from that of chlorophyll formation which takes place under such conditions even in the absence of light (**2, 17**).

Recent investigations (**4, 7, 12**) on the synthesis of vitamin A in land plants show that in the early stages of germination in the dark no vitamin is formed, but that after 10 to 16 days it is present in etiolated shoots. Light was found to accelerate the formation of the vitamin.

Vitamin production by unicellular algae (diatoms) has hitherto been observed (**1, 9**), but light was not excluded and the components of the nutrient medium were not limited to but one source of organic material, dextrose, for vitamin synthesis. An earlier paper (**18**) has demonstrated the production of vitamin A or its provitamin by a bacterium in darkness on a glucose-peptone substrate.[2] Even at the present time (**11**) vitamin A synthesis is associated by many people with the presence of light. In this paper the data do separate the factor of light from vitamin formation.

The well recognized ability of yeast to synthesize vitamin B (**13**) and G (**19**) has demonstrated that neither light nor pigment is essential in the

[1] This organism (no. 7 in our collection) was identified by Dr. Felix Mainx of the German University at Prague, Czechoslovakia. Pure cultures have been placed in the museum of Prof. E. G. Pringsheim of the same institution, from whom transfers may be obtained for a nominal sum.

[2] This has recently been confirmed by others. Jour. Biol. Chem. **103**: 339–351. 1933; Jour. Bact. **28**: 31–40. 1934.

formation of these factors. Vitamin B (old terminology) has been found in all species of bacteria and fungi tested (**20**). The presence of vitamin B has been investigated (**7**) in seeds, etiolated seedlings, and green plants, with the use of different light sources, with the resultant conclusion that no increase was obtained during germination and that the formation of vitamin B came at a later period during the development of the plant.

It early became known (**5**) that germinated seeds were strongly antiscorbutic. Light was shown to play no part in the formation of vitamin C (**3**). Later work indicated that oxygen was necessary for the formation of vitamin C (**8**), and that its concentration was increased by exposure to light (**7**).

Experimental work (18, 16, 6)

Pure cultures of the alga were grown upon a medium of the following composition (BEIJERINCK'S):

NH_4NO_3	0.5 gm.	$Fe_2(SO4)_3$	1 drop 1/10 saturated water solution
K_2HPO_4	0.2 gm.	Glucose (Difco)	10 gm.
$MgSO_4.7H_2O$	0.2 gm.	Agar-agar (Difco)[3]	15 gm.
$CaCl_2$	0.1 gm.	Distilled water	1000 cc.

Approximately 100 cc. of this medium were placed in 32-oz. medicine bottles and sterilized at 18 lb. pressure for 20 minutes. The bottles were then laid flat and the agar permitted to solidify, thus presenting as large a surface as possible. This medium was inoculated and the resulting algal growth harvested as already described (**6**). Methods for drying other than the one described could be devised to reduce opportunities for oxidation.

The flasks were incubated at room temperature in cupboards protected from light by piling the flasks in tiers on trays, the cotton-stoppered ends facing the tight-fitting wooden doors of the cupboards. There was no growth on inoculated control tubes of the same medium without sugar or agar which were placed in this same environment. This fact indicates that the darkness was complete and that the algae grown for the experiments led a completely saprophytic existence. Owing to the lengthy incubation period of five or six weeks, some cultures became contaminated and were discarded. Contaminants in the culture flasks were easy to detect, since bacteria or molds spread very rapidly over the surface during the protracted incubation period.

The algae were harvested using the method already referred to, and the

[3] The agar was further purified by washing in a continuous extractor for 48 hours with alcohol, then 24 hours with ether.

algal paste obtained was dried in petri dishes in a current of warm air. The algal mass was ground to pass through a 1-mm. sieve, and kept in a well stoppered bottle in a mechanical refrigerator. One hundred and fifty grams of dried algae may be obtained from five hundred culture flasks.

Extreme care was exercised in the growing, harvesting, and drying of the material to prevent bacterial growth; in fact, the only time bacterial growth could have occurred was while the material was being dried. This drying was complete enough for grinding within 36 hours, and the moisture content of the mass was lowered below that required for bacterial growth in much less time. By plating the material, it was found that the bacterial count was much less than that of the rations fed or of the drinking water remaining in the usual glass drinking fountains after the rats had contaminated them with food particles.

The rats used for testing the vitamin A content of the algae were the progeny of a colony which are maintained on a diet designed to minimize the amount of this vitamin the rat is able to store, but does supply a sufficient amount for growth, reproduction, and lactation. The general laboratory technique and methods for the preparation of food materials used in this investigation have been described (**14**) by others.

The compositions of the rations used in testing for vitamins A, B, and G are given in table I.

TABLE I

COMPOSITION OF RATIONS

	A-FREE*	B-FREE†	G-FREE‡
Casein	18.0	18.0	18.0
Salts§	4.5	4.5	4.5
Agar-agar	2.0	2.0	2.0
Butter fat	0.0	10.0	10.0
Crisco	10.0	5.0	5.0
Tapioca-dextrin	65.5	62.5	62.5
	100.0	102.0	102.0

* Vitamins B and G were supplied by 0.5 gm. dried yeast fed separately from the ration.

† Vitamin G was supplied by 0.5 gm. yeast which had been autoclaved 3½ hours at 15 lb. pressure and given separately from the ration.

‡ Vitamin B was suppliel in the form of tiki-tiki extract made according to the method of Williams, dried on dextrin, and fed separately.

§ McCOLLUM salt mixture, no. 185, to which had been added 0.8 per cent. $CaCO_3$ in each 100 gm. of ration.

Vitamin D was supplied in the B- and G-free rations by exposing the ration for 15 minutes, at distance of 2 ft., and in a thin layer, to a mercury vapor quartz lamp operating at 110 volts and 5 amperes.

Vitamin A

In testing the algae for vitamin A content, fourteen 28-day old rats were maintained on the vitamin A-free ration for seven weeks, when de-

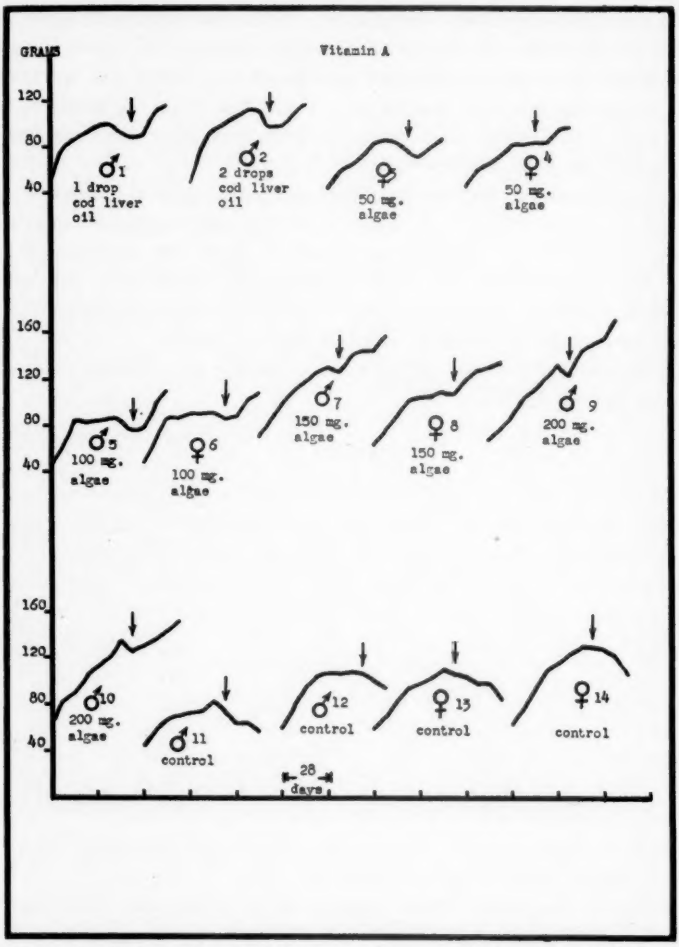

Fig. 1. Curative experiment, vitamin A: Arrows indicate point at which the feeding of the curative was started, except in the case of the controls, where they indicate the points at which the same number of weeks of experiment were reached.

cline in weight and xerophthalmia gave assurance of vitamin A deficiency. The rats were then divided into groups of two each, and different weights of the curative portions given as indicated in figure 1.

The growth curves of the rats receiving the algae as a curative show that 50 mg. of algae are inferior to the amounts of cod-liver oil fed in causing resumption of growth. With increasingly larger doses of algae there were correspondingly greater increases in the rate of growth. Three of the four rats which served as negative controls died from effects of the deficiency before the end of the experiment. The rats in the experiment were irradiated daily for two to five minutes to supply the vitamin D requirement.

Records of the food intake for the individual rats beginning at the time the curative portions were given show that the rats receiving 50 mg. and 100 mg. portions of algae ate less than an average of 40 mg. of ration per week, and those which received 150 mg. and 200 mg. of algae ate an average of 40 gm. per week.

Vitamins B and G

Seven rats, 28 days old, were maintained on the diet described, lacking both vitamins B and G. At the end of the eighth week the rats were placed in separately caged groups, and the supplement fed in addition to their ration. The yeast was fed in the form of pills while the algae was given in its powdered form in a carefully weighed portion for each rat. At the beginning of the experimental period the rats appeared to be suffering from inanition and malnutrition. From the manner in which these rats responded, both in weight and increased appetite, it could be concluded that the algae contained vitamins B and G.

Each of the vitamins was tested for separately, as indicated in the following experiments.

Vitamin B.—Three rats, 28 days of age, were maintained on a vitamin B-free basal diet throughout the experiment, as already explained. The rats were allowed to continue on this diet until avitaminosis was apparent. At the end of the fifth week, rats 1 and 3 were considered to be in a condition to warrant the addition of 0.5 gm. of algae to their diet daily. In the case of rat 2, it was not until the end of the twelfth week that the algae was fed.

Vitamin G.—Three rats, 28 days old, were maintained on the vitamin G-free ration. When the effect of the vitamin G deficiency was obvious, 0.5 gm. of algae was fed to each rat daily.

From the results obtained (fig. 2) it was concluded that vitamin B and G were present.

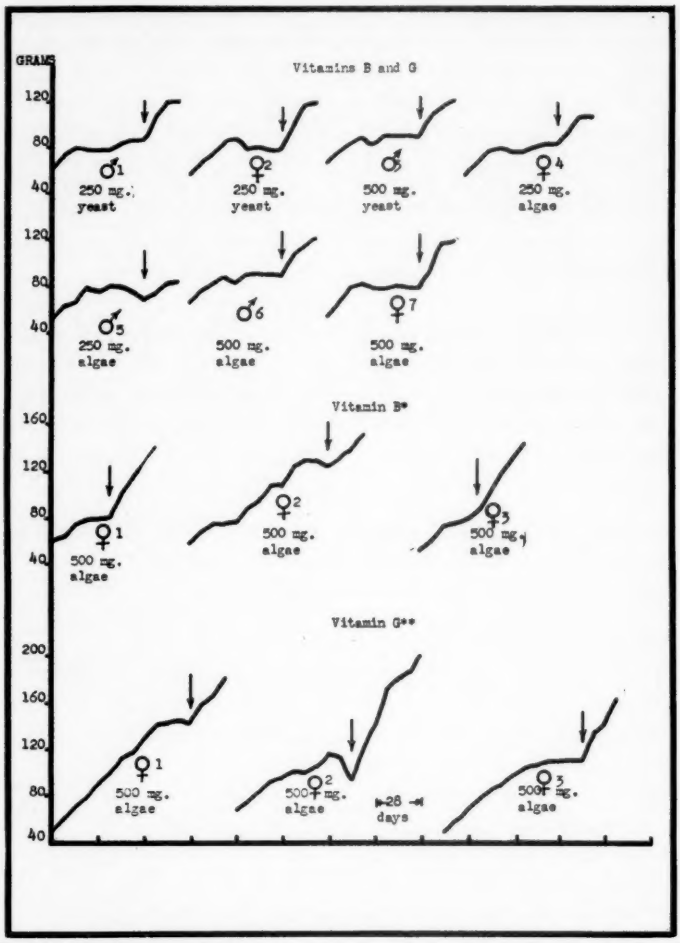

FIG. 2. Curative experiments, vitamins B and G, vitamin B, vitamin G: Arrows indicate point at which feeding of curative was begun.

* 0.5 gm. of autoclaved yeast was fed daily to supply vitamin G.

** 0.5 gm. of tiki-tiki (dried) was fed daily to supply vitamin B.

VITAMIN C

Six guinea pigs were fed a scurvy-producing ration which consisted of:

Ground yellow corn	3 parts
Ground rolled oats	3 parts
Oil meal	1 part
$CaCO_3$	1 per cent. of the ration
NaCl	1 per cent. of the ration

Prairie hay was also given *ad lib.* The algae which were used as the antiscorbutic had not been dried. The algal growth was removed from the agar by adding a small amount of water to the culture flask and shaking the flask after loosening the growth from the agar with a scraper. The resultant suspensions were centrifuged. The supernatant fluid from the centrifuge cups was used repeatedly, during the few hours required to make a harvest, to wash the algae from other bottles. Thus as little liquid as possible was used in making the harvest. The final liquid was of a syrupy consistency and had a moisture content of 90.7 per cent. The product was kept in an efficient refrigerator.

Two guinea pigs were fed 3 gm. each of the algal suspension daily, equivalent to 0.29 gm. of dried alga; two were given 5 cc. each of tomato juice; two guinea pigs were not given any supplement and served as negative controls. Other guinea pigs were kept on a complete diet to serve as replacements in case of accidents. When it was observed that 3 gm. of algae did not prevent the appearance of scurvy, the dosage was increased to 6 gm. Deaths from scurvy occurred in the animals fed algae as well as in the negative controls. Six gm. of algae are equivalent to 0.56 gm. of dried material, which is a small dose.

Discussion

These experiments indicate how certain plant processes and syntheses may be studied under known conditions by use of a plant behaving normally in darkness as well as in light and growing in pure culture under laboratory conditions. The failure of the plant to synthesize a particular vitamin does not necessarily signify that this organism is unable to synthesize the vitamin in nature, where a different nutrition may be available.

The results of the vitamin A experiment have a special interest, since the curative material was derived from a population long grown in complete darkness, for the inoculum per culture was not more than 5000 algal cells, and these multiplied in darkness until countless numbers of progeny resulted, which at no time during their growth had the added factor of illumination to be accounted for in the experiment. In the case of the etiolated seedlings, however, the seeds were produced in sunlight in the field. Also the hypothesis of SCHERTZ (**15**) that vitamin A is formed from pyrrole-ringed compounds found in soil cannot be substantiated, since none of the ingredients of our substance contained any substance remotely related to such compounds. Carotene was undoubtedly present (**10**) in the algae, however.

The positive results obtained in the experiments on vitamins B and G are not surprising. The organism belongs to the phylum Thallophyta,

comprising the algae and fungi. It is well recognized that fungi are able to synthesize large amounts of vitamin B in darkness, and it is also a commonly accepted but broad conception that fungi are algae devoid of chlorophyll. The organism used resembles a fungus in that it is able to exist saprophytically on organic media, and therefore fulfills an important attribute of a fungus but differs radically because of its power to manufacture chlorophyll in light or in darkness.

No data have been found relative to the vitamin C content of naturally occurring algae. It would be surprising if these plants in their natural environment did not synthesize this vitamin, which is universally present in succulent green plants.

Summary and conclusions

1. A unicellular green plant, *Chlorococcum* sp., synthesizing chlorophyll in complete darkness and able to exist saprophytically, was studied. Any synthesis of vitamins must of necessity have been elaborated by the cells without light, bacterial symbiosis, or complex nutrients. Such synthesis was effected from inorganic salts with a pure carbohydrate, dextrose as the only source of energy.

2. Vitamin A or its provitamin was shown to be synthesized in large quantities under these conditions. Dosages much smaller than those previously reported by workers using etiolated seedlings were more potent than the amount of seedlings fed.

3. Vitamins B and G were likewise found, but no vitamin C could be detected in the quantity of algae fed as supplement.

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PREPARATION OF AQUEOUS EXTRACTS OF SOLUBLE NITROGEN FROM PLANT TISSUES[1]

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Introduction

It has been shown by CHIBNALL (**3**), and by TOTTINGHAM *et al.* (**9**), that the drying of plant tissues before extraction changes the proportional distribution of nitrogen among the nitrogenous constituents. Accordingly, only aqueous extracts from fresh plant tissues have been used by CHIBNALL (**2**), VICKERY and PUCHER (**10**), NIGHTINGALE (**7**), and MURNEEK (**6**) in studies on the nitrogen metabolism of plants. Their results have shown that the various nitrogenous fractions included in the aqueous extracts are indicative of the course of nitrogen metabolism in plants.

The relative advantages of the solvents commonly employed in the preparation of plant extracts have been discussed by other investigators (**4, 5**). Hot alcohol used in concentrations of 50 to 80 per cent. has been recommended because it stops enzymatic action quickly, precipitates some or all of the proteins, and is a good solvent for the relatively simple nitrogenous compounds. APPLEMAN and MILLER (**1**) found that extracts of potato tubers prepared by the use of hot alcohol yielded practically the same amounts of non-protein nitrogen as did cold-water extracts. Hot water will destroy enzymes about as rapidly as will hot alcohol, however, and it is a slightly better solvent for the simple nitrogenous constituents than is the latter. By the use of hot water, cell membranes are killed quickly and thereby rendered permeable to the soluble constituents. Furthermore, the use of hot water simplifies the subsequent analytical procedure, since certain nitrogen determinations can be made only with alcohol-free samples (**4**).

In the preparation of aqueous extracts of soluble nitrogen, TOTTINGHAM *et al.* (**9**) and NIGHTINGALE (**7**) ground the plant tissues in a mortar with the aid of sand. The preparation of extracts by this method is attended with much labor and requires considerable time. This in turn limits the number of samples that can be extracted within a given period. Moreover, the time required for the preparation of an extract in this manner permits a loss of nitrogen or a change in the distribution of the nitrogenous fractions in plants containing such unstable compounds as some of the cyanogenetic glucosides (**8**).

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

It appears, therefore, that a method of extraction by boiling the tissue in water, somewhat similar to that used by VICKERY and PUCHER (**10**), would be rapid and might be applicable for use with plants containing unstable nitrogenous compounds. The development of such a method of extraction, and the testing of its applicability in comparison with the method now rather generally employed, have been the chief purposes of this investigation.

Finely minced plant tissue was divided into representative aliquots, some of which were boiled in water and then extracted, while others were ground in a mortar with sand before extraction. Quantitative comparisons were made of the commonly separable soluble nitrogenous fractions contained in samples prepared by the two methods.

Methods of extraction

In the preparation of an extract by the method recommended by TOTTINGHAM *et al.* (**9**), a 50-gm. or 100-gm. aliquot of finely minced or shredded plant material was ground in a large mortar with the aid of washed quartz sand, but without the use of ether as a plasmolyzing agent. The ground tissue was then transferred quantitatively on to a piece of "longcloth" and washed thoroughly with successive applications of water, each of which was removed by wringing with the hands. The aliquot was ground again in the mortar and then washed repeatedly on the same piece of cloth until the extract comprised a volume of about 950 cc. For convenience, this procedure for the preparation of extracts will be referred to as the grinding method.

The method of extraction by boiling is briefly as follows: A 50-gm. or a 100-gm. aliquot of finely minced plant tissue was placed in a beaker, covered with boiling water, and boiled on a hot plate for 20 minutes. The contents of the beaker were then transferred to an 18-inch square of longcloth, washed with successive applications of water, and extracted by wringing with the hands. After the tissue was washed four or five times, it was transferred to a mortar in which any large pieces of tissue were pounded to a pulp without the use of sand. This was easily accomplished, since the boiling treatment softens the tissue. The sample was then returned to the cloth directly, or, in the case of the cotton and peach samples, it was boiled for 10 minutes in a beaker and then transferred to the cloth. The washing and wringing were continued until the extract comprised a volume of 950 cc. This complete procedure will be referred to as the boiling method.

Peach stems are very woody and therefore were difficult to mince with a knife, but they were cut easily to shreds in a pencil sharpener. Short pieces that could not be cut in a pencil sharpener, and also the non-rigid, slightly lignified stem tips, were minced with pruning shears. The basal portions of nearly mature cotton stems are likewise woody, but they were

minced conveniently by the use of a large knife. Because of the woody and bulky nature of peach and cotton stems, it was thought advisable to boil these tissues for 10 minutes after they were pulped in a mortar. The need for the second boiling treatment has not been determined experimentally for plant materials so woody as these, but the data presented in tables II and III show that a second boiling treatment is unnecessary in the extraction of corn and tomato tissues.

The coagulable material, which was always proportionately small in amount when this method of extraction was followed, was precipitated by boiling the extract for one minute after the addition of 2 cc. of 10 per cent. acetic acid. The hot extract was then filtered through nitrogen-free filter pulp in a Buchner funnel. The filtrate was made to a volume of one liter and aliquots of this were used for the quantitative estimation of the nitrogenous fractions present.

Comparison of results

Duplicate 50 gm. samples of cotton and peach tissue were extracted by the two methods just described. A quantitative comparison of the nitrogenous constituents extracted from the samples by each method is shown in table I. It is evident from these comparisons that the two methods are equally efficient in removing the soluble nitrogenous constituents from the plant material.

TABLE I

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH COTTON AND PEACH TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	COTTON STEMS AND PETIOLES		PEACH STEMS	
	BOILING	GRINDING	BOILING	GRINDING
	%	%	%	%
Total N in tissues	0.339	0.339	0.504	0.504
Total soluble N	0.172	0.174	0.127	0.121
Amide N	0.025	0.025	0.013	0.013
Humin N	0.011	0.012	0.007	0.006
Basic-free amino N	0.035	0.035	0.015	0.016
Basic N	0.038	0.038	0.032	0.030
Cyanogenetic N	.....	.....	0.045	0.039
Ammonium N	0.008	0.008	0.013	0.014
Nitrate N	0.031	0.031	0.000	0.000

It is also evident that there is no difference in the nitrogenous composition of extracts prepared by these methods except in the case of the peach, which loses cyanogenetic nitrogen during the process of grinding. Such a

loss, however, may be anticipated when peach stems, leaves, or roots are ground and extracted for half an hour or more previous to a boiling treatment which stops enzymatic action. On the other hand, when peach tissue is minced quickly, covered immediately with boiling water and boiled for 20 minutes, no appreciable loss of this form of nitrogen takes place except in the case of rapidly growing twigs.

In order to obtain a further comparison of these methods of extraction, two representative 100-gm. aliquots of well minced corn stems were boiled for 20 minutes and then extracted as previously described. Two other aliquots from the same lot of stems were extracted by the grinding method. The extracts were analyzed, and the results are shown in table II. It is apparent that the boiling method, as well as the grinding method, gives consistent results when duplicate aliquots are extracted, and that variations in the composition of extracts from different samples are not due to the method of extraction. The data in table II also show that a single 20-minute period of boiling is adequate for the extraction of the soluble nitrogenous constituents from samples that are not very woody.

TABLE II

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH CORN TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	Corn stems				Corn leaves	
	Boiling		Grinding			
	Number of aliquot				Boiling	Grinding
	A	B	C	D		
	%	%	%	%	%	%
Total N in tissues	0.174	0.174	0.174	0.174	0.705	0.705
Total soluble N	0.108	0.108	0.107	0.109	0.112	0.120
Amide N	0.002	0.003	0.003	0.003	0.002	0.002
Basic-free amino N	0.013	0.014	0.014	0.014	0.025	0.026
Basic N	0.017	0.017	0.016	0.015	0.037	0.039
Ammonium N	0.006	0.005	0.005	0.005	0.003	0.004
Nitrate N	0.077	0.076	0.075	0.075	0.051	0.050

In another test, triplicate aliquots were taken from a well mixed sample of finely minced tomato stems and leaves. One of the aliquots was extracted by the grinding method previously described. Another was boiled 20 minutes before extraction according to the procedure just outlined. The third aliquot was boiled for 5 minutes, extracted by washing and wringing several times, then returned to fresh boiling water and boiled again for 5 minutes. After a second extraction similar to the first, the boiling was repeated and the extraction completed by further washing and wringing. The results appear in table III and show that a single boiling

treatment for 20 minutes, followed by thorough washing and wringing, was just as effective in the removal of the soluble nitrogenous compounds from the tissue as was repeated boiling and extraction. Furthermore, the results show that both of the boiling methods of extraction were slightly more effective in the removal of soluble nitrogen than was the grinding method.

TABLE III

NITROGENOUS FRACTIONS IN AQUEOUS EXTRACTS OF FRESH TOMATO TISSUES. NITROGEN EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

	Composite of stems and leaves		
	Single boiling	Repeated boiling	Grinding
	%	%	%
Total N in tissues	0.447	0.447	0.447
Total soluble N	0.207	0.206	0.197
Amide N	0.006	0.006	0.005
Total amino N	0.036	0.035	0.035
Ammonium N	0.009	0.009	0.010
Nitrate N	0.103	0.101	0.098

From the results of this investigation it is evident that the preparation of aqueous extracts of the soluble nitrogenous constituents from plant tissues by the boiling method here described is equally as effective as is the preparation of these extracts by the grinding method (9) using quartz sand in a mortar, and is much more economical of time and labor. Furthermore, this method limits the possibility of a loss of nitrogen, and of a change in the composition of the extract with respect to the nitrogenous constituents.

Summary

1. Two methods for the preparation of aqueous extracts of the soluble nitrogenous constituents from plant tissues have been compared.

2. It was found that the preparation of extracts by a process of boiling for 20 minutes, followed by thorough washing through "longcloth," was just as effective in the removal of soluble nitrogenous constituents as was extraction by the process of grinding in a mortar with sand, followed by thorough washing through the cloth.

3. The preparation of aqueous extracts by the boiling method is easier and quicker than by the grinding method.

4. The loss of cyanogenetic nitrogen which takes place when peach extracts are prepared by the grinding method may be minimized by the use of the boiling method.

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RELATION OF CERTAIN AMINO ACIDS TO CARBON DIOXIDE AND MYCELIUM PRODUCTION OF *FUSARIUM OXYSPORUM*

ARTHUR K. ANDERSON AND KATHRYN EMMART

(WITH ONE FIGURE)

Introduction

It has been known for many years that the ingestion of foods, especially proteins, stimulates metabolism in animals. LUSK (**11, 12**) has shown that certain amino acids produce the same stimulation as proteins. He believes that the stimulatory effect of proteins can be accounted for by the active amino acids which they contain. LUSK (**10**) has shown that glycine, alanine, leucine, and tyrosine stimulate oxidation in the order given, while glutamic acid does not. ATKINSON and LUSK (**3**) added aspartic acid and asparagin to the list of those which showed no effect. RAPPORT and BEARD (**15**) found phenylalanine to have the greatest stimulatory effect of any amino acid. ORT and BOLLMAN (**14**) found that cystine, glycine, alanine, phenylalanine, leucine, histidine, and valine catalyzed the action of hydrogen peroxide on dextrose, while glutamic acid, aspartic acid, and tyrosine had no effect.

With regard to the effect of amino acids on plant metabolism there is a considerable literature. In green plants SPOEHR and MCGEE (**17**) noted that the leaves of *Helianthus annuus* when fed a glycine solution consumed glucose more rapidly, and showed a greater respiratory activity as measured by carbon dioxide evolution, than leaves not so fed. KOSER (**9**) observed that certain amino acids supported growth of some bacteria but not others. GORDON and MCLEOD (**6**) noticed no marked effect of amino acids on hardy bacterial organisms such as *B. coli*, but that they stimulated the growth of certain delicate organisms. Several workers (**8, 13, 20**) have shown that certain amino acids influence the amount and size of yeast cells and also yeast activity in fermentation.

With regard to the effect of amino acids on fungi, SCHULZE (**16**) has shown that leucine and tryosine serve as a food material for *Penicillium glaucum*. TITS (**18**) found that leucine, glutamic acid, histidine, cystine, and glucosamine stimulated the germination of spores of *Phycomyces nitens*. HERZOG and SALADIN (**7**) have reported a decided stimulation in the metabolism of *Penicillium glaucum*, as measured by carbon dioxide production, after the addition of leucine to the medium.

In this laboratory considerable work has been done on the metabolism of *Fusarium oxysporum*. ANDERSON, EVERITT, and ADAMS (**2**) have shown that the main products of metabolism of this organism, when grown in a medium in which glucose is the only source of carbon, are carbon dioxide and ethyl alcohol. Since *Fusarium oxysporum* grows well in a medium containing no organic nitrogen, it was felt that this organism offered an excellent opportunity to study the stimulatory effect of amino acids on fungus metabolism. This paper reports a study of the rate of metabolism of *F. oxysporum* in glucose media containing glycine, leucine, tyrosine, and aspartic acid as measured by carbon dioxide production. Glycine, leucine, and tyrosine were selected as amino acids which might be expected to produce a stimulation in metabolism and the aspartic acid as one which might not.

Methods

The culture of *Fusarium oxysporum* used was obtained from the Division of Plant Pathology at the University of Minnesota. The stock medium was that used by ANDERSON (**1**) in his work on *F. lini* and had the following composition:

Ammonium nitrate	1.00 gm.
Monopotassium phosphate	0.50 "
Magnesium sulphate	0.25 "
Glucose	20.00 "
Water to make	1000 cc.

Using this as a basis, the amino acid media were prepared by adding 0.5 gm. carbon equivalent of an acid for each 300 cc. of stock medium (the amount used in each culture flask), *i.e.*, 1.5635 gm. of glycine, 0.9105 gm. of leucine, 0.8385 gm. of tyrosine, and 1.386 gm. of aspartic acid. Two controls were run, control A being the stock solution alone and control B being the same as control A except that 0.5 gm. of carbon in the form of glucose (1.25 gm.) was added to each flask. Three hundred cc. of each medium were placed in 500-cc. pyrex Erlenmeyer flasks. Triplicate experiments were run in each case. Each flask was closed by a two-hole rubber stopper through which extended two glass tubes. Cotton plugs were placed in the outer end of each tube to prevent contamination.

After autoclaving at 15 pounds' pressure for 20 minutes and cooling, each flask was inoculated with 10 cc. of spore and mycelium suspension. The stoppers were sealed with paraffin and the ends of the glass tubes were closed with rubber tubes and screw clamps.

Separate samples of all solutions were autoclaved with the culture flasks and, after cooling, their pH values were determined by the electrometric

method using a quinhydrone electrode. During the experiment the flasks were kept on laboratory desks at room temperature.

Carbon dioxide determinations were made at frequent intervals by aeration into TRUOG (**19**) towers containing barium hydroxide solution. The excess barium hydroxide was then titrated with 0.1 N hydrochloric acid using phenolphthalein as an indicator.

The cultures were allowed to grow for 229 days, at which time two sets, those containing glycine and aspartic acid, were opened as it was planned to terminate the experiment at that time However, the others were then allowed to grow longer, 270 days in all. After opening, the cultures were immediately filtered through Gooch crucibles and the mycelia washed, the washings being discarded. The mycelia were dried to constant weight at 100° C. The filtrates were used for the determination of pH and of residual glucose by the method of FOLIN and WU (**5**).

Presentation of data

Figure 1 and table I present the results of this investigation. Figure 1 shows the total amount of carbon dioxide produced during growth. This amount is the average of triplicate determinations for each culture medium.

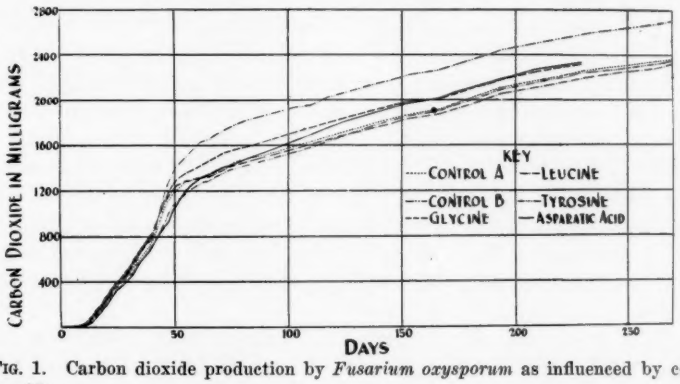

FIG. 1. Carbon dioxide production by *Fusarium oxysporum* as influenced by certain amino acids.

After a determination, the weight obtained from each culture was added to that previously obtained, so that the amount given for any day represents the total carbon dioxide produced from the time of inoculation to that day. It can be observed that control B, the medium modified by the addition of glucose, produces the greatest amount of carbon dioxide, which indicates that the carbon in glucose is the best source for carbon dioxide production by the fungus. The curve for the carbon dioxide produced by the fungus on the medium modified by aspartic acid is below all the others until the

53rd day, after which it gradually rises until on the 229th day it is above all except that for control B. The high initial acidity of this medium evidently causes the early retardation of metabolism, and, as the pH rises, a more optimum condition is reached, thereby producing a greater activity.

It can be observed that the glycine, as well as the aspartic acid medium, gave more carbon dioxide than control A. These two amino acids are therefore used as a carbon source by *Fusarium oxysporum*, although not to the same extent as an equal carbon equivalent of glucose. Tyrosine slows the rate of carbon dioxide production, while leucine has the greatest retarding effect. The comparisons are best made at 229 days, but by observing the chart one can see that the same ratio would exist if the curves for glycine and aspartic acid had been continued.

Table I gives a résumé of this investigation. The figures given represent the averages of triplicate determinations. It is unfortunate that the period of growth of the cultures containing glycine and aspartic acid was not so long as that of the others. It is therefore difficult to make comparisons. One can observe that the weights of mycelia obtained from the two cultures are even greater than that from control B in which growth was continued over a longer period of time. This might be accounted for by the beginning of autolysis when the fungus is allowed to grow after most of the glucose is consumed. On the other hand, since tyrosine produces a slightly greater weight than control B and leucine only a slightly smaller one, it appears that the amino acids studied, with the exception of leucine, are utilized to a greater extent than an equal carbon equivalent of glucose to produce fungus body.

The small amounts of residual glucose noted in the table show that most of the 6 gm. of glucose originally present in each flask is utilized during growth. The high values obtained for the cultures containing glycine and tyrosine are not significant because these two amino acids were found to have a slight reducing action on the reagents used in the glucose determination.

In every culture the hydrogen-ion concentration decreases during growth. The lowest original pH is for the media containing aspartic acid. This is due to the fact that this amino acid is a dicarboxylic acid. For the other media, the range of pH is from 4.6 for control B to 5.3 for that containing tyrosine. The final pH of all the media is 7.5 or above. Comparisons are again rather difficult on account of the difference in length of the period of growth of the cultures. The greatest increase in pH is noted for the medium containing aspartic acid, a change from 3.1 to 8.0. The medium containing glycine had a final pH of 8.1. As previously stated, these two amino acids, although the fungus was allowed to grow on them for a shorter length of time, produce the greatest weight of myceliun, and

TABLE I

TOTAL CARBON DIOXIDE AND MYCELIUM PRODUCTION, RESIDUAL GLUCOSE, AND ORIGINAL AND FINAL pH OF THE MEDIA

DESCRIPTION OF MEDIA		PERIOD OF GROWTH	WEIGHT			pH	
			CARBON DIOXIDE	DRY MATTER IN MYCELIUM	RESIDUAL GLUCOSE	ORIGINAL	FINAL
			mg.	*mg.*	*mg.*		
Controls	A: stock medium alone	270	2366.0	376.3	12.7	4.7	7.7
	B: stock medium plus glucose	270	2713.6	406.3	20.6	4.6	7.6
Media modified by addition of amino acids	Glycine	229	2322.4	483.8	37.6	4.8	8.1
	l-leucine	270	2321.9	403.1	28.8	4.9	7.7
	l-tyrosine	270	2351.6	410.9	37.1	5.3	7.5
	l-aspartic acid	229	2341.0	563.0	20.0	3.1	8.0

up to the time of the opening of the cultures, the greatest amount of carbon dioxide with the exception of control B.

Conclusions

At the outset of this experiment it was expected that certain amino acids which are known to produce a stimulation of metabolism in animals might do so with the fungus, *Fusarium oxysporum*. It is apparent from figure 1 that there is no such stimulatory effect as measured by carbon dioxide production. Cultures containing glucose in amounts equivalent in carbon content to the carbon of the added amino acids show just as rapid a production of carbon dioxide as those containing amino acids. In the end cultures containing added glucose produce much more carbon dioxide than do any of the cultures containing amino acids. Aspartic acid and glycine are utilized by the fungus for the production of carbon dioxide, while leucine and tyrosine retard the production of carbon dioxide.

All of the amino acids studied are utilized by the fungus in the production of mycelium. With the exception of leucine they are all a better source of mycelium building material than glucose. Aspartic acid is outstanding in its ability to produce mycelium.

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RESPIRATORY RATE AND ENZYME ACTIVITY AS RELATED TO THE HARDENED CONDITION OF PLANTS[1]

S. T. DEXTER[2]

(WITH ONE FIGURE)

Introduction

In several papers in the literature (**8, 3**), reference is made to the dormancy of plants or their rate of respiration, in connection with the hardened condition. ÄKERMAN (**1**) has shown that hardy varieties of winter wheat tend to retain larger stores of sugars throughout the winter than tenderer ones. NEWTON and ANDERSON (**6**) have followed the rate of respiration of several varieties of winter wheat throughout the growing season and found that the respiratory rate of each variety decreased as the season progressed, with the hardiest variety showing the lowest output of carbon dioxide per gram of dry matter. The implication in at least some of this work is that the hardened condition is accompanied by a lowered rate of respiration and "dormancy." NEWTON and BROWN (**7**) have studied the activity of catalase in the expressed sap of winter wheat varieties, but their results were difficult to interpret and highly variable.

Since it is possible to grow plants in such a way that hardening will not occur at a low temperature in the dark (**4, 9**), and equally possible to grow them so that hardening will occur, it seemed desirable to measure the carbon dioxide output from samples of plants so stored, and to determine the catalase and oxidase activity of the plants before and after exposure to the low temperature.

Methods

Duplicate samples (of about 15 plants each) of winter wheat from sand cultures were placed in flasks on damp asbestos fiber. Tests by the freezing-exosmosis method of DEXTER *et al.* (**4**) on plants from the same pots showed that hardening did not occur in the cold room (2° C.) in the dark. The flasks were stored in a room kept constantly at 2° C. for 24 hours before the measurements of carbon dioxide output were started. Compressed air was passed through a soda-lime tube, and through barium hydroxide solution, thence into the flasks, and into modified Pettenkoffer tubes containing tenth-normal barium hydroxide. Before the measurements the flasks were flushed out with carbon dioxide-free air. The passage of air through the

[1] Contribution from the Plant Physiology Laboratories, University Farm, University of Minnesota.

[2] National Research Council Fellow.

flasks was at a rate of about 15 cc. per minute, and was kept constant throughout the experiment by means of a bubble-counter. The blank on the apparatus for a run of 24 hours was 0.3 cc. of base neutralized. The total output of carbon dioxide from the plants in 24 hours at 2° C. was measured at the intervals indicated in table I. At the expiration of the experiment, the two samples were dried to constant weight, ashed, and the results computed on the basis of milligrams of carbon dioxide output per gram of organic matter in the sample. The experiment was repeated on another set of winter wheat plants from the same variety (Minhardi), and these results are included in the table. According to tests, plants from this second set did not harden in the dark at 2° C. In a similar way, the experiment was run with cabbage plants which were high in starch and which were shown to harden markedly during exposure to the low temperature.

Plants from the same sets of pots were tested for catalase and oxidase activity according to the following procedure. Before making the first analysis for catalase or oxidase, all plants were held in darkness at 2° C. for 24 hours. They were then divided into two sets, one of which was given continuous illumination, the other held in darkness. The cabbage plants hardened excellently whether in darkness or in light. The two sets of winter wheat failed to harden in darkness but hardened well in the light. After a period in the cold room, all samples were again held in darkness for 24 hours before samples were taken, in order to bring them to a condition relatively comparable to that at the beginning of the experiment. Each sample of winter wheat consisted of six plants, two from each of three pots. Cabbage samples consisted of 10 discs, 1 cm. in diameter, from one side of the leaves of an individual plant. After a period in the cold room, corresponding punches were made on opposite sides of the leaves. The determination of catalase followed that of LANDON (5). The green weight of the samples was determined, an equal weight of precipitated chalk was added (in the case of the wheat samples an arbitrary weight, 0.5 gm.), and the samples were quickly ground, with the addition of a pinch of quartz and a few drops of distilled water, to a smooth paste. The sample was then further diluted with water until fifty times the weight of the sample had been added. This aqueous suspension was stirred with an electric agitator while aliquots were withdrawn. Two cc. of sample were used in each determination of catalase. Duplicates rarely differed as much as 5 per cent. and usually were identical. Five cc. of solution were used in the determination of oxidase, with 10 cc. of 2.5 per cent. hydroquinone.

Table I gives the amount of CO_2 respired in 24 hours by 1 gm. of ash-free plant substance in the dark at 2° C. over a period of several days. This shows that there was a continuous decrease in respiration in each of the five samples. This seems somewhat remarkable in that the physiological condi-

TABLE I

RESPIRATORY RATE PER GRAM ASH-FREE DRY MATTER DURING CONTINUOUS EXPOSURE AT 2° C. FOR SEVERAL DAYS. VALUES ARE IN MILLIGRAMS CO_2 IN 24 HOURS

DAYS AT 2° C. BEFORE TESTED	SAMPLE				
	WHEAT 1 NON-VEGETATIVE (−N)		WHEAT 2 VEGETATIVE (+N)		CABBAGE
	a	b	a	b	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	17.68	.....	7.36	.....	15.88
2	.....	.....	.....	.....	8.37
3	.....	8.14	.....	.....	7.41
4	.....	.....	.....	7.42	5.82
5	.....	.....	.....	.....	5.19
6	6.53	.....	.....	.....	4.07
7	.....	.....	5.53	.....	3.96
8	.....	5.39	.....	.....	3.71
9	.....	.....	.....	5.40	.....
10	5.56	.....	.....	.....	.....
11	.....	.....	.....	.....	.....
12	.....	.....	.....	.....	.....
13	.....	.....	5.37	.....	.....
14	.....	.....	.....	4.96	.....

tions of the plants were very different. Sample 1, wheat, was very non-vegetative (−N), since nitrogen had been withheld for a period in the greenhouse. It contained no starch, but was high in sugars. It did not harden in the cold room. Sample 2, wheat, was highly vegetative (+N), and also failed to harden in the cold room in the dark. The cabbage sample was high in starch and relatively low in sugar at the beginning of the experiment, according to analysis on similar tissue, and at the end of the experiment was decidedly higher in sugar (about 30 per cent.) and lower in starch. Similar cabbage plants hardened well under the conditions of the experiment. In analyses of similar wheat samples, it was found that the sugar decreased materially during storage in the dark, whereas it increased in the case of the cabbage. No essential difference is evident in the trend of the respiration in either case. Furthermore, whether the plants hardened

or failed to harden, and whether the sugar percentage increased or decreased, a similar repression of respiration during continuous storage at the low temperature is most evident. BARKER (2) has recently noted a similar depression in the rate in respiration during long-continued storage of potatoes at a low temperature, even though the percentage of sugar was increasing greatly.

Figure 1 presents the data of table I in graphic form.

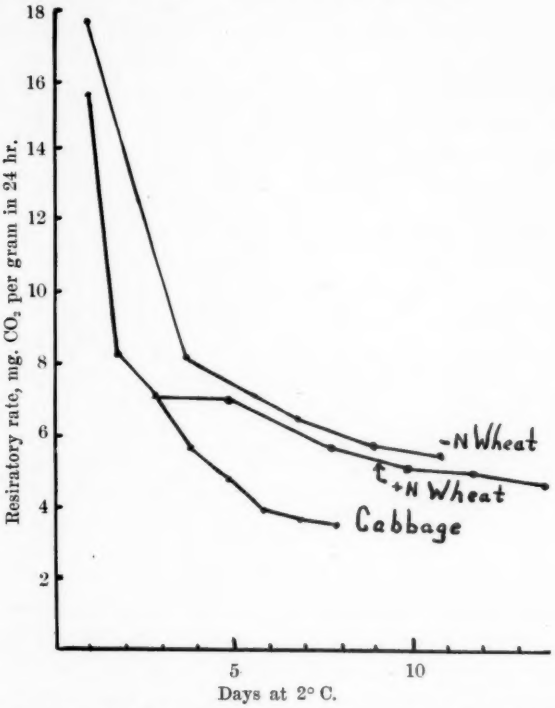

FIG. 1. Data of table I in graphic form: Respiratory rate of all samples decreased over a period of several days at 2° C. regardless of hardening or increase in sugars.

In table II are given the results of the determinations of catalase activity in cabbage and winter wheat plants which were subjected to continuous cold. The value for catalase is the volume of oxygen set free in 5 minutes. The oxidase activity was determined on the same samples. The average change in oxidase activity during exposure for 5 days to 2° C. for the first four samples of cabbage was from 3.0 cc. to 2.8 cc. For the second set of

four samples, from 3.1 cc. to 3.0 cc. This change is less than the blank with distilled water. The changes with winter wheat were, similarly, within the experimental error.

TABLE II

CATALASE ACTIVITY OF CABBAGE AND WINTER WHEAT PLANTS BEFORE AND AFTER EXPOSURE TO LOW TEMPERATURES (2° C.). VALUES ARE IN CC. OF OXYGEN LIBERATED FROM HYDROGEN PEROXIDE IN 3 MINUTES BY A SAMPLE OF 0.04 GM. GREEN WEIGHT

DAYS AT 2° C. BEFORE TESTED	OXYGEN SET FREE IN 5 MINUTES' REACTION WITH HYDROGEN PEROXIDE							
	CABBAGE PLANTS				WHEAT PLANTS			
	IN LIGHT		IN DARK		IN LIGHT		IN DARK	
	+N	−N	+N	−N	+N	−N	+N	−N
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	4.4	5.0	3.8	7.2	11.7	10.2	11.8	11.3
6	4.5	4.7	4.6	7.4	8.9	9.2	13.4	15.3
11	.....	.....	.....	.....	7.4	8.6	15.5	18.3
Repeated all dark								
1	7.8	8.6	8.8	10.0	.....	.....	.....	.....
6	7.4	9.1	9.5	9.1	.....	.....	.....	.....

From table II it would appear that the catalase activity of cabbage plants does not change perceptibly in either the light or the dark as a result of exposure to continued cold, during which time marked increase in resistance to freezing injury has developed. The change in the first four samples was from 5.1 to 5.3 cc.; in the case of the second set of samples, the averages were 8.8 and 8.78 cc.

The winter wheat plants which received continuous light, thereby increasing markedly in percentage dry matter and in hardiness, showed an unmistakable decrease in catalase activity. Those plants which were stored in the dark showed an increase in catalase activity. If the values were computed on a dry matter basis, rather than on a green weight basis, these differences would be still more pronounced. There is an evident difference therefore in the behavior of the two species used, since the cabbage showed virtually no change in catalase activity in either case. There was, however, a marked increase in percentage dry matter in the case of the wheat which was illuminated during storage. This relatively large amount of sugar and other storage material might be low in catalase. The cabbages, to the contrary, were already high in dry matter, and heavily stored with starch when put in the cold room. At the end of the experiment there happened to be a

slightly greater dry matter content in those cabbages which were stored in the dark than in those which had received light. Probably not much photosynthesis took place in these already high-carbohydrate plants.

There is a notable lack of correlation between change in respiratory rate and catalase or oxidase activity during exposure to low temperatures. For, although respiratory rates decreased during storage at 2° C. in vegetative and non-vegetative wheat plants and in high-carbohydrate cabbage plants, the oxidase activity of none of them changed perceptibly during this period. The catalase activity of both types of wheat increased (in the dark) while that of the cabbage plants remained constant. The lack of correlation between change in respiratory rate and change in hardiness is equally noticeable. In all cases, whether increase in hardiness took place or not, a seemingly identical behavior was noted in the decreasing respiration of the samples during the exposure at 2° C. If the same mechanism is at work here that BARKER (**2**) has described in the case of potatoes (which do not harden materially), it would appear that a "respiratory depressant" is produced regardless of increase in sugar or increase in hardiness.

Summary and conclusions

1. Cabbage and winter wheat plants were examined for rate of respiration and catalase and oxidase activity during exposure to a low temperature (2° C.).

2. Respiratory rates of all samples continuously decreased during storage at this temperature, regardless of increase in percentage sugar in the tissues or increase in hardiness.

3. Oxidase activity appeared unchanged as a result of such storage, and was not seemingly correlated with rate of respiration, with increase in sugars, or with change in hardiness.

4. Catalase activity in cabbage was not changed by storage at 2° C. for five days, either in the light or in the dark, although in both cases the plants hardened markedly and showed large increases in percentage of sugars.

5. Catalase activity decreased in winter wheat plants which were illuminated in the cold room, and increased in those stored in the dark. According to the interpretation of the writer, this change should not be associated with the differences in hardiness which developed under those two conditions of storage. It seems more likely that the increase of storage material in the plants in the light may be responsible for the decrease in catalase activity, since storage tissue in the crown was found much lower in catalase activity than leaf-blade tissue on the same plants.

6. Although hardy varieties of winter wheat probably have a lower respiratory rate than tender varieties (**6, 1**), we have no evidence to show that the hardened condition as such is especially characterized by a low

respiratory rate. Plants which have been stored at a low temperature appear to suffer great depressions in respiratory rates whether they harden or not.

7. It seems necessary to correlate more clearly our ideas of dormancy, rest period, and cold resistance of plants.

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METHOD FOR THE PREPARATION OF GREEN PLANT MATERIAL FOR THE EXTRACTION OF JUICES[1]

L. D. DONEEN

Introduction

The extraction of plant juices is often desirable or essential for a study of their physico-chemical properties. As methods in general use are laborious and time consuming, it was found desirable in a recent investigation on the nutrition of wheat to develop a rapid, simple, yet accurate method for extracting the plant juice.

A review of the literature indicated that several methods have been devised for the treating of plant tissue to facilitate the expression of its juice. These methods may be divided into two general groups: either the material is ground in a mortar or grinding mill, or it is frozen in a slushy mixture of ice and salt, a cold storage room, liquid air, or solid carbon dioxide. The method in most frequent use is freezing the tissue in a test tube or jar for a number of hours, thawing it rapidly, and subjecting it to pressure. Some excellent and comprehensive reviews of the methods employed have been contributed by MEYER (**6**), GORTNER (**1**), SAYRE and MORRIS (**8**), and GREATHOUSE (**3**).

In the proposed method the material was heated in an autoclave. Approximately 100 young winter wheat plants were used, some grown in the greenhouse and others under natural field conditions of late winter and early spring. Juices were satisfactorily obtained from the wheat plants at regular intervals during the growing period.

Procedure

The wheat plants were wrapped in damp cloths in the field, placed in a moist, air-tight container, and brought immediately to the laboratory. The wrapped plants were then placed in an autoclave for five minutes at 15 pounds' pressure, although a reasonable variation in the length of time does not affect the results. Material which was not wrapped tightly was often sufficiently cooked in one minute at 15 pounds' pressure, but it was found advisable to leave the tightly wrapped material in the autoclave for five minutes or until the thoroughly cooked plant tissue was light brown in color. After autoclaving, the sample was removed and cooled to room temperature. According to the results of a number of trials, the moisture content of the tissue does not seem to be affected materially by autoclaving.

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The small amount of condensation that may occur during the heating was lost by evaporation while cooling to room temperature. The sap was expressed as soon as the material was cool to prevent further loss of moisture and possible changes in percentage of the plant juice.

A large percentage of the juice might have been expressed by hand, but to be certain of obtaining a representative sample, a hydraulic press was employed, applying uniform pressures of 7000 pounds per square inch and uniform times of draining of four minutes after this pressure was reached. Approximately 90 per cent. of the plant juices was extracted by this method. However, this varies with the type of plant, its age, and the conditions under which it is grown.

The method of freezing the wheat plants in a cold storage room at a temperature of 30° C., and the method of autoclaving them antecedent to expression of the sap were compared for determinations of the freezing point, total solids, total sugars, and nitrate-nitrogen of the expressed plant juice.

The freezing point of the juice was determined in the usual freezing point apparatus with a Beckmann thermometer. The figures reported were the results of four closely agreeing determinations on each sample. The freezing point depression readings were converted into equivalent osmotic values according to the equation of LEWIS (**5**).

The percentage total solids in the expressed plant juice was obtained by the Abbé refactometer according to GORTNER and HOFFMAN (**2**).

The total sugar content was determined by the Munson-Walker method, the cuprous oxide being weighed directly according to the recommendation of the Association of Official Agricultural Chemists (**7**).

The nitrate-nitrogen content was found by the colorimeter method of HOLTZ and LARSON (**4**).

Results

A comparison of the results for a number of determinations of osmotic pressure and total solids, obtained by the proposed autoclave method and freezing method antecedent to expressing the plant juice, is given in table I.

Samples 1, 2, 3, 4, and 5 were grown in the greenhouse during the winter. Samples 6 and 7 were grown on land continuously cropped by wheat and were sampled in the field in the early spring. Samples 8 and 9 were similar to samples 6 and 7 except that they were grown on summer fallow land.

A comparison of the total sugar and nitrate-nitrogen of the expressed juice as obtained by the two methods is given in table II.

The osmotic pressure, total solids, total sugar, and nitrate-nitrogen of the autoclaved and frozen plant tissue agree closely. The small variation

TABLE I

OSMOTIC PRESSURE AND TOTAL SOLIDS OF PLANT SAP OBTAINED BY AUTOCLAVING AND BY FREEZING THE PLANT MATERIAL ANTECEDENT TO EXPRESSING THE SAP

SAMPLE NO.	WHEAT VARIETY	METHOD OF TREATMENT	FREEZING POINT DEPRESSION	OSMOTIC PRESSURE		TOTAL SOLIDS	
				ATMOSPHERES	DIFFERENCE	PERCENTAGE	DIFFERENCE
			° C.				
1	Hybrid 128	Frozen	1.062	12.78	−0.10	7.7	−0.20
		Autoclaved	1.054	12.68		7.5	
2	White Odessa	Frozen	0.908	10.96	−0.11	5.4	+0.30
		Autoclaved	0.900	10.85		5.7	
3	Coppie	Frozen	1.120	13.48	−0.25	6.25	+0.35
		Autoclaved	1.099	13.23		6.6	
4	Hussar	Frozen	0.940	11.32	+0.55	5.25	−0.20
		Autoclaved	0.986	11.87		5.05	
5	Hybrid 128	Frozen	0.953	11.48	−0.19	4.90	+0.15
		Autoclaved	0.938	11.29		5.05	
6	Ruddy	Frozen	0.861	10.37	+0.11	9.25	−0.10
		Autoclaved	0.870	10.48		9.15	
7	Hybrid 128	Frozen	0.800	9.60	0.00	8.80	−0.30
		Autoclaved	0.802	9.60		8.50	
8	Ruddy	Frozen	0.688	8.28	+0.16	5.70	+0.05
		Autoclaved	0.701	8.44		5.75	
9	Hybrid 128	Frozen	0.658	7.91	−0.21	5.40	0.00
		Autoclaved	0.648	7.80		5.40	

that occurred could be accounted for partly by the difference in sampling. The proposed treatment of plant tissue can therefore be satisfactorily used for some phases of plant juice study instead of the freezing method.

SAYRE and MORRIS (**8**) showed that the sugar content of tissue could be calculated from the sugar content of the expressed juice. A comparison of the percentage sugar and nitrate-nitrogen in the plant juice from the autoclaved material with that of the dried wheat plant is given in table III.

The tissue was dried at a temperature of 60° C. in an air current. There were smaller differences between the duplicate autoclaved samples than between samples of dried tissue. This is especially true of sugar content.

Numerous investigations have shown that drying of green plant material has a tendency to result in a change of the various carbohydrates and proteins. This is well illustrated in table III.

The autoclave method cannot be used in a study of proteins, that is, proteins precipitated by heat, any more than the freezing method can be used in such a study. This method, however, has many advantages for the study of the truly soluble material not affected by heat. For instance, the

TABLE II

TOTAL SUGAR AND NITRATE-NITROGEN OF PLANT SAP OBTAINED BY AUTOCLAVING AND BY FREEZING THE PLANT MATERIAL ANTECEDENT TO EXPRESSING THE SAP

SAMPLE NO.	WHEAT VARIETY	METHOD OF TREATMENT	TOTAL SUGAR		NITRATE-NITROGEN	
			PERCENTAGE	DIFFERENCE	PERCENTAGE	DIFFERENCE
			%		%	
3	Coppie	Frozen	0.41	−0.02	0.157	−0.004
		Autoclaved	0.39		0.153	
5	Hybrid 128	Frozen	0.41	+0.01	0.141	+0.004
		Autoclaved	0.42		0.145	
6	Ruddy	Frozen	3.75	−0.10	0.018	+0.001
		Autoclaved	3.65		0.019	
7	Hybrid 128	Frozen	3.60	+0.05	0.009	+0.002
		Autoclaved	3.65		0.011	
8	Ruddy	Frozen	0.78	+0.04	0.041	−0.003
		Autoclaved	0.82		0.038	
9	Hybrid 128	Frozen	0.65	+0.03	0.032	−0.002
		Autoclaved	0.68		0.030	

enzyme action is almost instantly stopped by the high temperature in the autoclave. A large proportion of the plant juice is extracted and consequently a more representative sample of plant sap is obtained. This is ap-

TABLE III

PLANT SAP AND DRIED WHEAT TISSUE COMPARED FOR DETERMINATIONS OF TOTAL SUGAR AND NITRATE-NITROGEN BASED ON DRY WEIGHT

VARIETY	PLANT TREATMENT	PERCENTAGE SUGAR			PERCENTAGE NO_3–N		
		SAMPLE		DIFFERENCE	SAMPLE		DIFFERENCE
		1	2		1	2	
Ruddy	Sap autoclaved	6.02	6.11	0.09	1.02	0.98	0.04
	Tissue dried	6.33	5.60	0.73	0.88	0.92	0.04
Little Club	Sap autoclaved	4.88	4.88	0.00	1.04	1.01	0.03
	Tissue dried	4.88	3.17	1.71	0.90	1.10	0.20
Jones Fife	Sap autoclaved	7.35	6.93	0.42	0.86	0.86	0.00
	Tissue dried	6.02	4.88	1.14	1.04	1.02	0.02

parently due to a better breaking down of the membranes and cell walls by the heat. The method is rapid as it is possible to autoclave and express ten samples of plant tissue per hour.

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NUCLEAR DIVISION IN *TRADESCANTIA VIRGINIANA*

BEATRICE LEHMAN AND F. M. ANDREWS

Introduction

The study of *Tradescantia virginiana* L. as reported in this paper is possible only during its period of anthesis. This lasts, in this region, from about May to October, varying somewhat with the season. The blooming period in this plant may, however, be somewhat extended by employing suitable cultural and cutting methods. Even under the most favorable conditions, the period during which it is possible to study the behavior of the nucleus in the cells of the hairs on the staminal filaments is a comparatively short part of the year.

Tradescantia virginiana has been an object of study for many years. A brief summary of this study has recently been given by KÜSTER (**2**). Although many problems have been solved by work on *Tradescantia virginiana,* still others remain for investigation. Among these may be mentioned: (1) The possible occurrence of intercalary division in the staminal hairs; (2) the position of granules on the protoplasmic strands in the cells of the staminal hairs; (3) chromosome kinetics; (4) BECKER'S (**1**) oxidation processes and nuclear division; (5) the effect of chemicals on nuclear division, etc.; and (6) questions of diapedesis. TELEZYNSKI'S statement that the cells of the staminal hairs remained living for eight days in paraffin oil also certainly merits further investigation.

The following points have been investigated and briefly recorded in this study of nuclear division in *Tradescantia virginiana,* namely, the effects of temperature, carbon dioxide, ether, chloroform, iodine, and fluorescein. In some cases the effects on the protoplasm of *Elodea canadensis* has been mentioned for the sake of comparison.

Experimentation

EFFECTS OF TEMPERATURE ON NUCLEAR DIVISION

The lower temperatures employed in this work were regulated by the use of a MOLISCH freezing box containing dry ice, and the medium and higher temperatures were regulated by using a thermostat, or a heating stage, according to requirements. Table I presents the average results obtained with ten specimens studied for each of the different temperatures recorded. Reading downward the figures in each column of the table indicate the time required for each stage in the nuclear division, and the total time for the nuclear division at the given temperature is recorded at the foot of each column. No divisions occurred at 18° C. The various "stages"

here recorded are the same stages of nuclear division as were figured by STRASBURGER (3).

Table I shows that a temperature of 28° C. was the most favorable, in the stages mentioned, causing nuclear division in 69 minutes, which is 11 minutes less than the total time of the stages recorded by STRASBURGER. Even at 27.5° C. the nucleus divided somewhat more slowly (taking 5 minutes longer, on the average) than indicated by STRASBURGER'S stages. A very interesting variability is shown in the length of time required for completion of nuclear division, beginning with no division at all at 18° C. and rising abruptly to 340 minutes at 19° C. With increasing temperature the time required decreases to 69 minutes at 28° C., which was the optimum average temperature for the specimens studied. Above 28° C. the nuclear divisions proceeded more and more slowly until at 40° C. 330 minutes were required, almost the same period of time as was required at 19° C. Above 40° C. no divisions occurred.

When the temperature is raised or lowered gradually the division of the nucleus is not so noticeably affected as when there is a sudden rise or fall. If the cell is heated to 35° C. suddenly the division is slow or ceases entirely. When the temperature of the oven which was 29° C. was raised in 15 minutes to 38.5° C. nuclear division took place slowly. When heated to 41° C. the nucleus did not divide nor did it recover if it had begun to divide. At a temperature of 18° C. or below, no division occurred, nor was there apparent recovery. It was found that the protoplasm of *Tradescantia virginiana* can remain active at a temperature higher than that which will allow nuclear division. If the temperature has not been too high nor too low, the protoplasm will resume movement after a return to normal conditions.

EFFECTS OF CARBON DIOXIDE

A few observations were made to determine the effects which CO_2 has upon nuclear division of *Tradescantia*. We have measured, first, the length of time required to stop nuclear division; second, the length of time CO_2 can be permitted to act and the nucleus still recover and continue division; and third, the length of time required to kill the nucleus with CO_2.

Carbon dioxide was applied for periods of 20, 10, 5, and 1 minute, and 30, 15, and 10 seconds to different cells whose nuclei were in the process of division. In each case pure moist fresh air at the proper temperature was drawn over the specimens containing the dividing nuclei for 30 to 60 minutes after the application of the CO_2. On the average the nuclear division stopped almost instantly on the application of pure CO_2 or within 2 to 4 seconds, which simply indicates the length of time necessary for the CO_2 to penetrate the cell wall and protoplasm and to reach the nucleus. In treated cells with dividing nuclei, the nuclei failed to recover, except where the CO_2 was applied to the dividing nucleus of the staminal hairs for only 10 sec-

TABLE I

TIME (IN MINUTES) BETWEEN STAGES OF NUCLEAR DIVISION IN RELATION TO TEMPERATURE

STAGES	TEMPERATURE																
	18° C.	19° C.	20° C.	21.5° C.	22.5° C.	24° C.	25.5° C.	26° C.	27.5° C.	28° C.	29° C.	31° C.	32.5° C.	35° C.	37.5° C.	40° C.	41° C.
Stage 3–4	0	40	30	25	20	18	14	12	10	8	10	15	20	25	30	40	0
Stage 4–5	0	40	30	25	20	18	14	12	10	8	10	15	20	25	30	40	0
Stage 5–6	0	20	15	11	13	10	9	7	5	3	5	10	10	15	20	30	0
Stage 6–7	0	20	15	14	13	12	9	7	5	3	5	10	10	15	20	30	0
Stage 7–8	0	20	15	14	13	12	9	7	5	3	5	10	10	15	30	30	0
Stage 8–9	0	40	30	25	20	18	14	12	10	8	15	20	20	25	30	40	0
Stage 9–10	0	80	60	45	40	28	24	22	20	18	25	30	40	45	50	60	0
Stage 10–11	0	80	60	45	40	28	24	22	20	18	25	30	40	45	50	60	0
Total	0	340	255	204	179	144	117	101	85	69	100	140	170	210	260	330	0

onds. In this case, recovery was apparent in 10 minutes after fresh moist air was drawn over the cell. The nucleus was killed by an application of pure CO_2 for 15 seconds.

After an application of CO_2 for 10 minutes, the protoplasm of the young leaf of *Elodea* recovered in 3 minutes; but after an application of CO_2 for 30 minutes there was no recovery of the protoplasm in the cells of the young leaf of this plant, even after fresh moist air was drawn over the specimen for 25 minutes.

Effects of ether

The effect of ether upon the protoplasm and the nuclear division of the staminal hairs of *Tradescantia* and upon the protoplasm of the leaf of *Elodea* was ascertained by the following experiments.

The vapor from a saturated solution of ether in distilled water was drawn over a cell of *Tradescantia virginiana* containing a dividing nucleus; it continued to divide for 5 to 7 minutes and then stopped. When fresh moist air was then drawn over the specimens they recovered in 15 minutes.

After the application of ether vapor for 30 minutes, the nucleus of a cell of *Tradescantia* which had stopped dividing recovered under the influence of fresh moist air in 20 minutes. It is obvious that ether vapor is less destructive to the nuclear division of plants than CO_2.

A cell of *Tradescantia* with a dividing nucleus was placed for 15 minutes over strong ether in a ring cell on a slide; 30 minutes were required for recovery after this treatment, although in some cases even a 5- to 10-minute exposure was sufficient to kill such a dividing cell under these conditions.

Elodea frequently recovered immediately after having been treated with strong ether vapor by the ring-cell method for 15 minutes. On the other hand, as much as 1 hour or more was often required to kill the cell when thus subjected to strong ether vapor.

When the hairs from a staminal filament of *Tradescantia* showing nuclear divisions, and a leaf of *Elodea,* both of which showed active protoplasmic movement at the beginning, were placed side by side in a saturated solution of ether water in amounts sufficient to cover them, the nuclear division in *Tradescantia* was stopped in 30 seconds and the movement of the protoplasm of *Elodea* in 2 minutes. No recovery occurred in either case. Ether is especially harmful to nuclear division and protoplasmic movement when in direct contact with the cells. The vapors, however, are not so harmful, and the cells may even recover some time after its application.

Effects of chloroform

When a current of air was drawn through distilled water saturated with chloroform all nuclear divisions in the staminal hairs of *Tradescantia* were stopped in 60 seconds and there was no recovery. Actively moving protoplasm in the cells of the leaves of *Elodea* was stopped in 5 minutes after

the chloroform vapor was admitted; and even after 15 minutes, the use of fresh moist air failed to bring about its recovery.

One-minute submergence in water saturated with chloroform was sufficient to kill the protoplasm of *Elodea*; and nuclear division in *Tradescantia* was stopped instantly when the cells were submerged in strong chloroform water.

Effects of iodine

The effects of iodine upon the nuclear division of cells of staminal hairs of *Tradescantia* and on the protoplasmic movement of the cells of *Elodea* were investigated.

The vapor from a 5 per cent. solution of iodine was drawn over specimens of *Tradescantia* and *Elodea* for 30 minutes. The vapor had no effect on either, even in 1.5 hours. When, however, specimens of *Tradescantia* and *Elodea* were placed directly in a 5 per cent. solution of iodine, the cells were immediately stained brown, nuclear division and protoplasmic movement stopped at once, and the cells could not be made to recover. Twelve parts of 5 per cent. iodine solution in 500 parts of water stopped division, and the cells were stained deep yellow at once. The same strength of iodine acting on protoplasm of *Elodea* caused rapid decrease in its movement, and after 20 minutes it was completely stopped. No recovery occurred. One cc. of 5 per cent. iodine in 1 cc. of distilled water stopped the protoplasmic movement instantly and no recovery took place.

Specimens of *Tradescantia* with dividing nuclei and leaves of *Elodea* with actively moving protoplasm were placed on a glass ring cell containing a saturated aqueous solution of iodine. This stopped the division and apparently killed the nucleus almost at once. The protoplasm of leaf cells of *Elodea* stopped moving in 5 minutes and did not recover.

Effects of fluorescein

A beautiful green solution of fluorescein was obtained by placing several grains of fluorescein in distilled water. The dividing nuclei of staminal hairs of *Tradescantia,* mounted in this solution, were stained yellow within 5 to 10 minutes. The dye had no effect on the nuclear division and all of the various stages could be readily followed.

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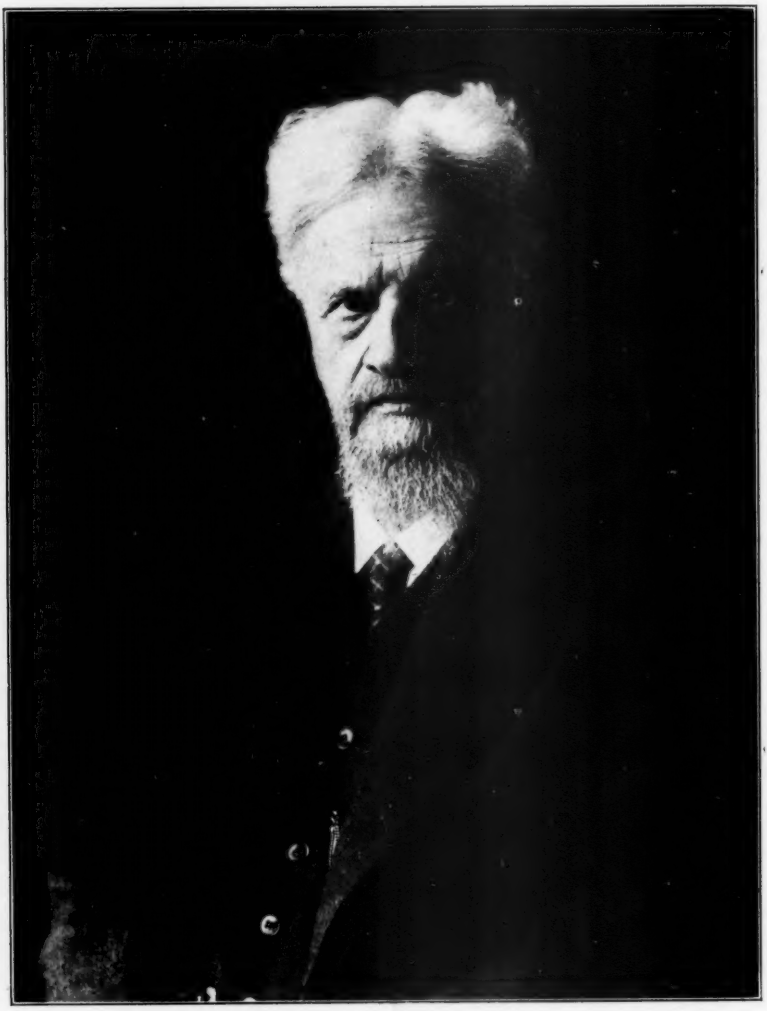

GOTTLIEB HABERLANDT
1854

BRIEF PAPERS

GOTTLIEB HABERLANDT
1854

(WITH PLATE III AND ONE FIGURE)

On November 28th of this year a great German botanist of the older generation will be eighty years old. The writer of this short biographical sketch was his student and it is with a feeling of sincere admiration that he thinks of the life and work of his great teacher.

GOTTLIEB HABERLANDT was born under the Austro-Hungarian monarchy at Ungarisch-Altenburg, where his father taught classes in natural sciences at the government agricultural school. The father's interest in biology as well as his talent for drawing and music were inherited by his son.

In 1873 HABERLANDT entered the University of Vienna and was much attracted by JULIUS WIESNER's lectures on the anatomy and physiology of plants. In 1874 his first botanical paper, about cellulose in cork tissues, appeared in the "Oesterreichisch botanische Zeitschrift."

HABERLANDT was not very much influenced by JULIUS WIESNER but he admired him and appreciated his teacher's friendly interest. While a student in Vienna it was the reading of the works of C. NÄGELI, W. HOFMEISTER, HUGO VON MOHL, and FRANZ UNGER, but especially of JULIUS SACHS, through which HABERLANDT trained himself. As a student he published several papers, among which are an investigation of lenticels and a study of the winter coloring in perennial leaves. The last named paper was also accepted as a thesis for the Ph.D. degree which he obtained in 1876. In the autumn of 1877 he went to SIMON SCHWENDENER in Tübingen. HABERLANDT had already conceived the idea of a physiologic interpretation of plant anatomy which SCHWENDENER had suggested in "Das mechanische Prinzip im anatomischen Bau der Monokotylen." In Tübingen HABERLANDT undertook his first independent investigation, "Die Entwicklungsgeschichte des mechanischen Gewebesystems."

SCHWENDENER recognized in HABERLANDT his most talented pupil and later chose him to be his successor in the chair of general botany at Berlin.

HABERLANDT started his research work with a very definite program. He intended to investigate, in the course of years, one system of plant tissues after another and to incorporate finally these investigations into a handbook of physiological plant anatomy. He decided to start with the chlorophyll bearing photosynthetic system, because it was typical for plants and no danger existed here of his being misled by false analogies with animals.

HABERLANDT'S method was to reach conclusions about the relations between structures and functions of tissues on the basis of purely anatomical investigations, and his early deductions were confirmed by later experiments carried out by A. F. W. SCHIMPER.

During the autumn of 1878 HABERLANDT was admitted to a docentship in botany at the University of Vienna. In 1880 he received a call to the Technische Hochschule in Graz, in Austria. There he was made acting professor of botany and simultaneously he became privatdocent at the University.

HABERLANDT was very happy in the beautiful alpine city of Graz with its lovely surroundings and proximity to the Adriatic Sea. In the year following his appointment in Graz, HABERLANDT married CHARLOTTE HAECKER to whom he had become engaged in Tübingen. In 1884 he became professor extraordinarius and in 1888, after LEITGEB'S death, ordinarius at the University.

The first investigation which HABERLANDT undertook in Graz was on the apical growth of the phanerogams. He emphasized the function of the apical cell, which he interpreted through the formation of segments, in contrast to SACHS who defined the apical cell as merely a gap in the constructive system of the cell walls of the vegetative point. After having studied the assimilatory system, HABERLANDT intended to take up the conductive system including the vascular bundles. He took up first the vascular bundles in the petioles of ferns and second the latex tubes which he explained as having the function of typical conductors for the assimilation products of plants.

Meanwhile SCHWENDENER had been called to Berlin and had founded his Botanisches Institut. There his pupils worked out problems in the field of physiological plant anatomy. These papers appeared without a very definite program and HABERLANDT realized that a fundamental classification of tissue systems from the viewpoint of physiological plant anatomy was urgently needed. He decided to write his handbook without waiting until all the necessary Vorarbeiten should be completed. In 1884 appeared the first edition of the "Physiologische Pflanzenanatomie."

The "Physiological Plant Anatomy" saw six editions and is still much read while contemporary text-books of plant physiology are no longer in use. In his charming memoirs, "Erinnerungen, Bekenntnisse und Betrachtungen" (Berlin, Julius Springer, 1933), which every botanist should read, HABERLANDT expressed the hope that some day somebody else might give an independent treatment of evolutionary physiological anatomy of plants (Entwicklungphysiologische Anatomie) as a counterpart of the functionally physiological anatomy which he formulated in his handbook.

HABERLANDT's "Physiologische Pflanzenanatomie" found many readers throughout central, northern, and eastern Europe and in Japan. It was translated into English but never became very popular in France.

It was in the winter semester, 1893–94, that the author of this biographical sketch first attended HABERLANDT's lectures on physiological plant anatomy at the University of Graz. I have a very vivid recollection of my teacher. His lectures were extremely inspiring and beautifully organized. A natural talent for drawing, as well as the magnificent anatomical and flower models prepared by his technician, H. GASSER, added to their instructiveness. In the microscope room he visited every advanced student several times a day, giving advice, suggesting new viewpoints, explaining new ideas. He had eminently what the Germans call a philosophical mind, never being satisfied with the establishment of mere facts, but always attempting a theoretical interpretation.

As professor ordinarius of botany and director of the botanical institute and garden, HABERLANDT enjoyed a great deal of independence in administering his small domain. In matters of budget he dealt directly with a department head in the provincial governor's office who in turn merely represented the imperial and royal secretary of education. HABERLANDT was twice elected dean of the faculty of philosophy in the University but declined election to the office of rector magnificus.

The other professor of botany, CONSTANTIN VON ETTINGSHAUSEN, whose assistant I was, did not always cultivate the best of personal relations with HABERLANDT. My position was therefore a very delicate one but HABERLANDT always treated me in a very friendly and considerate manner. I am proud to be one of his Schüler. During his 44 years of active teaching in Vienna, Graz, and Berlin, HABERLANDT must have had hundreds of advanced students. Only six of them, including the writer of this paper, reached university positions, and of these six two are dead, EDUARD PALLA and CARL ERICH CORRENS.

At this time fell also a visit to the botanical garden of Buitenzorg in Java (1891–92). Fruits of this trip were studies of the transpiration of plant leaves and a charming volume "Eine botanische Tropenreise," which had three editions and is still a very popular book among botanists, owing to HABERLANDT's broad scientific observations and artistic presentation and to his masterful pencil sketches which accompany the text.

In 1909 SCHWENDENER retired, at eighty years of age, from the chair of plant physiology at the University of Berlin and he suggested that HABERLANDT be his successor. The latter began his lectures in Berlin in the autumn of 1910.

With a heavy heart HABERLANDT left his beautiful Botanisches Institut in Graz and the lovely alpine city with his many congenial friends. He

had not accepted the call to Berlin until he had been promised a new institute for plant physiology with greenhouses and gardens, but it was three years before HABERLANDT could move his institute to Dahlem where he also had his official residence.

HABERLANDT worked at that time with cell division hormones, wound hormones, necro-hormones, lepto-hormones, etc. He also started a serial publication for his own and his students' contributions called "Beiträge zur allgemeinen Botanik." The Beiträge did not continue after the second volume owing to the unfavorable financial conditions in Germany. HABERLANDT gave normally during autumn and winter at Dahlem a course in anatomy and physiology of plants. This was changed later to physiological plant anatomy, when the professor extraordinarius of general botany was giving a lecture course on plant physiology.

During the summer semester HABERLANDT gave what he called "Grundzüge der Botanik." This was a survey course touching, in the short span of three months, four times a week, on cytology, anatomy, organography, physiology, and the taxonomy of thallophytes, bryophytes, pteridophytes, and spermatophytes. This course was held at the University building in the city of Berlin.

HABERLANDT would have liked to add to his "Physiologische Pflanzenanatomie" chapters on the anatomy of reproductive organs as well as on the evolutionary physiology of tissue systems. Another wish was to write a text-book of botany for artists and craftsmen. His own paintings in water colors and oil had splendidly prepared him for such a task. He was rarely qualified to do it. But soon after he had organized his new Pflanzenphysiologisches Institut in Dahlem the world war broke out. His four sons served in it, two in the German and two in the Austrian army. All returned unharmed. HABERLANDT's first wife had died in February of 1910 and he married again in August of 1914. He had seven children, five by his first wife and two were born during the war from his second marriage.

During the world war HABERLANDT served on various food boards and after peace had returned he continued his botanical researches, producing valuable papers to date. In a recent letter he assured the writer of this biography that his eyes are still as serviceable for microscopic work as they were thirty years ago. May he be spared for a long time, for the benefit of botany, and reach the nineties as did his great teacher, SCHWENDENER.

HABERLANDT is one of a great group of German botanists. Of the others, NÄGELI, SACHS, DE BARY, STRASBURGER, SCHWENDENER, ENGLER, CORRENS, PFEFFER, WIESNER, KERNER, and WETTSTEIN have all passed away. HABERLANDT and the 78-year old HANS MOLISCH are the only survivors of that group of brilliant botanists whose race was German and who belonged to Germany, Austria, and Switzerland. His is a good illustration of the best

type of German university professor; a thorough scientist, an excellent teacher, a man of broad scientific and humanistic interests, upright and independent in his convictions. The old German universities had many men of this type and it is to be hoped that they also may be found in the new generation.

Perhaps it was the humanistic education that created the intellectual leaders among the scientists of HABERLANDT'S generation. The gradual disappearance of this background may account for the smaller number of brilliant men of scientific thought among the much larger masses of scientifically trained men in our time. The greatest change in the history of civilization is the disappearance of classical education. It has ruled the intellectual life of European mankind from the time of the Renaissance to the world war. HABERLANDT still belongs to the intellectual aristocracy of the classical era.—A. C. NOÉ, *University of Chicago.*

FIG. 1. Institute for plant physiology and anatomy, University of Graz, Austria.

DEMONSTRATION OF SOME OF THE MECHANISMS INVOLVED IN THE ASCENT OF SAP IN PLANTS

(WITH ONE FIGURE)

The usual demonstration of osmosis with sugar solution separated from water by a differentially permeable membrane is unsatisfactory as a model representing the plant mechanisms involved in sap ascent. Beginning students are often confused because in the demonstration they see a solution rising in a tube due to a force exerted not from above, as it is in the plant, but from below. A simple rearrangement of the materials ordinarily used provides a very convincing demonstration of the process of osmosis, and at the same time shows more exactly how osmosis in the leaf cell brings about the rise of water in the stem below. The addition of a capillary evaporating surface to the apparatus then makes the picture of the mechanisms involved and their coordination quite complete.

Figure 1 (A) shows how the apparatus is arranged. A glass tube (t), one or more meters long, communicating above with a short glass cylinder

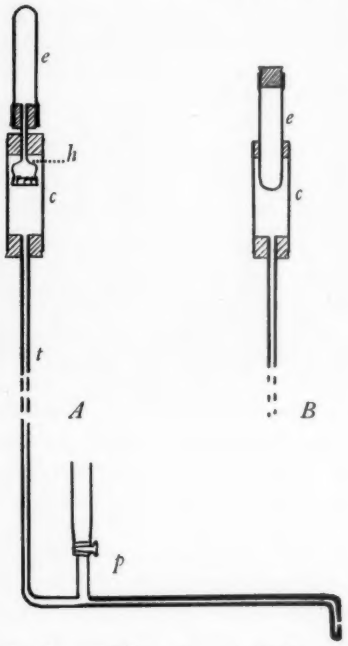

FIG. 1. Apparatus for demonstration of some of the mechanisms involved in sap ascent.

(dimensions about 5×20 cm.) is fastened upright, the lower end stoppered, and the whole filled with water, excluding all air bubbles. A membrane is tied over the bulb end of a short thistle tube (h), the tube filled with water, and sealed into the cylinder as shown. A small lump of copper sulphate in the water stains the membrane blue, and may protect it from decay. Before adding sugar the stopper below is removed. Water filters very slowly through the membrane, and the column of water in the tube (t) falls. Movement of the water may be measured conveniently by means of a potometer (p) attached below. Sucrose crystals are now dropped into the thistle tube from above (through a water-filled funnel attached to the stem of the thistle tube) with the result that the water immediately reverses its direction of flow. This rise of water, following as a result of merely adding a solute, affords an especially convincing demonstration of the power of osmosis.

If, now, a porous clay evaporating cylinder (e) be filled with water and fastened to the end of the thistle tube as shown in the figure, the rise of the water in the tube *as in the plant* will clearly be affected by surface and evaporational forces, as well as by osmosis. Subjecting the apparatus to hot, dry moving air will cause the water to move rapidly in the tube. Surrounding the evaporating surface with a humid atmosphere, on the contrary, will cause osmosis to work alone, and the solution will be forced out through the porous clay walls, superficially simulating guttation. When osmosis dominates over evaporation, the membrane is expanded to its maximum (as would be a leaf-cell wall and protoplast under these conditions) and bulges downward. When the rate of evaporation is very high, the membrane becomes slack or may even bulge upward. It is a good exercise for the student to explain the various observed rates of water movement in terms of the relative vapor tension in the water, solution, capillaries, and atmosphere, and clarifying to see the working together of the different processes involved in this simple model.

The tabulation (p. 858) gives data representative of the type of results that may be expected.

For more advanced students acquainted with the use of precipitated membranes in osmometers, a modification of this apparatus may be used. Here the porous cylinder alone serves as both the osmotic and the evaporating unit, thus approaching a step nearer the situation in the leaf cell where there may be no essential difference except in position between the absorbing and evaporating parts. Copper ferrocyanide is precipitated into the walls of the cylinder according to the usual procedure. After being filled with syrup or sugar solution, the porous cylinder is tightly stoppered and fitted into the water cylinder (c), so that only its lower half is immersed, the upper part being exposed to evaporation (fig. 1 B). A narrow rub-

CONDITIONS	RATE OF RISE PER MINUTE	OBSERVED RATE OF MOVEMENT IN CAPILLARY TUBE OF POTOMETER PER MINUTE
	mm.[3]	*cm.*
Before adding evaporating surface—osmosis alone	19.9	7.6
Evaporation plus osmosis. Quiet air	26.2	10.0
Evaporation plus osmosis. Hot, moving air	52.3	20.0
Osmosis. Evaporation prevented by moist towel and beaker	14.6	5.6

Membrane: pig bladder
Room temperature: 26°C.
Weight of sucrose: 50 gm.
Volume thistle tube: 32 ml.
Volume evaporating cylinder: 50 ml.
Total height of water column: 1.5 m.

ber ring fitted around the porous cylinder makes the seal between it and the water cylinder. This apparatus works as does the one previously described, except, of course, that it will not "guttate" unless the membrane is incomplete at the stoppered end.—ERNEST H. RUNYON, *92 Edgewood Ave., La Grange, Illinois.*

A NOTE ON FRUITING APPLE SPUR PHYLLOTAXY

The writer was considerably interested in HUBBELL'S paper[1] in the April, 1934, issue of PLANT PHYSIOLOGY on blind wood in roses, particularly in his observation that the fruitful shoots of the hybrid tea rose, Mme. Butterfly, averaged 7.3 nodes per shoot, while the unfruitful shoots averaged 4.9 nodes per shoot. In 1927 while at Oregon State College a nearly identical observation was recorded with respect to apple cluster bases. Usually the fruiting spurs showed 8 or 10 leaves below the terminal fruit stalks on the cluster base; 8 leaves were present in a phyllotaxy of $\frac{3}{8}$, or 10 leaves in a phyllotaxy of $\frac{2}{5}$. Spurs unfolding small numbers of leaves were seldom fruiting. The rule is not invariable, but the majority of cases adhere closely to it. Spurs of the Starking apple in the University of Wisconsin orchards this spring showed the arrangement beautifully. Incidentally, 8 nodes or leaflets on shoots of hybrid tea roses usually represent one complete phyllotaxy in $\frac{3}{8}$ arrangement. In the apple in strong fruiting spurs, *one complete*

[1] HUBBELL, D. S. Causes of blind wood in roses. Plant Physiol. 9: 261–283. 1934.

phyllotaxy of leaves is usually laid down on the cluster base below the terminal flower embryos. In cases where more than 8 leaves appear on the spur, the internode length[2] beyond the eighth node increases very rapidly, and fruiting decreases abruptly.—HAROLD L. COLBY, *University of Wisconsin, Madison, Wisconsin.*

[2] ROBERTS, R. H. Off year bearing and apple spur growth. Jour. Pomol. and Hort. Sci. **2**: 16–37. 1920.

NOTES

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists will be held at Pittsburgh, December 27–29, 1934. The William Penn Hotel has been selected as headquarters for the Society. Early reservations should be made, as the demand for accommodations may be heavy.

At this time, early in October, only a few facts about the meetings can be presented. A joint symposium on plant hormones is scheduled with the Botanical Society of America for Thursday afternoon; a joint session with the American Society for Horticultural Science has been arranged for Friday morning; and a joint session with section G, A. A. A. S., for Friday afternoon. There will probably be three other sessions for the reading of papers, the usual business meetings, and the annual dinner. Tickets for the dinner should be obtained promptly on arrival in order that proper arrangements may be made to accommodate everyone.

Life Membership Committee.—The committee whose duty it is to make the award of the CHARLES REID BARNES life membership in the American Society of Plant Physiologists has been appointed by President BURTON E. LIVINGSTON, who was the first CHARLES REID BARNES life member in 1926. The members of the committee are Dr. H. A. SPOEHR, of the Carnegie Institution of Washington, chairman, Dr. JAMES B. OVERTON, University of Wisconsin, and Dr. T. G. PHILLIPS, University of New Hampshire. The by-laws provide that the award is to be made to a plant physiologist from foreign lands every fifth year. The first such award may be made this year. The announcement of the award is made at the annual dinner.

Council Representation, A. A. A. S.—The two representatives of the American Society of Plant Physiologists in the Council of the A. A. A. S. appointed by President LIVINGSTON are Dr. CHARLES O. APPLEMAN, University of Maryland, and Dr. CHARLES A. SHULL, University of Chicago.

Exhibition of A. A. A. S.—The American Association's Exhibits Committee (Dr. F. C. BROWN, chairman) states that the annual science exhibition will this year be larger, better, and more comprehensive than heretofore. It will include many exhibits by research workers, as well as numerous exhibits of apparatus by commercial firms. Individuals and institutions are invited to correspond with Dr. BROWN concerning the exhibition of new research apparatus and techniques, preferably arrangements that show motion or visible changes, or which are otherwise generally

attractive. This is an opportunity for plant physiologists to give valuable service to their science, and to science in general.

Sixth International Botanical Congress.—The plant physiology section of the Sixth International Botanical Congress at Amsterdam will consider six great topics: Photosynthesis, phyto-hormones, oxidation-reduction and metabolism, permeability and accumulation of mineral elements, translocation of plastic materials, and influencing the cycle of development in plants. There will be a general paper on phyto-hormones, accompanied by several special papers. In connection with the translocation of plastic materials, the problem of the submicroscopic structure of cell walls will be considered. These topics indicate the broad fundamental character of the proposed sessions. All botanists who can do so should journey to Amsterdam in September, 1935, to share in the advantages and privileges of international cooperation in scientific progress.

Carotenoids.—Volume 31 of the Monographien aus dem Gesamtgebeit der Physiologie der Pflanzen und der Tiere is an interesting and valuable summary of our knowledge of the carotenoid pigments. The author is Dr. L. ZECHMEISTER, director of the Chemical Institute of the University of Pécs, Hungary. The major part of the work is devoted to the plant carotenoids; the chapter on animal carotenoids is very brief. It covers the historical development of research in this field, the chemical nature, physiology, and rôle of the pigments, their relationship to other chemical entities, methods of investigation of constitution, isolation, and quantitative estimation. A special section gives detailed information about each of the twenty pigments which have been found in plants. Most of these are polyene compounds containing oxygen. Two are hydrocarbons (carotene and lycopene), and all but a few belong in the C_{40} series of compounds. The work reflects and emphasizes the rapid progress made in the last five years in unraveling the different constitutional problems which the carotenoids present. The book is published by Julius Springer, Berlin, at RM 28 for brochure style, and RM 29.40 for cloth-bound copies.

Respiration.—The appearance of a posthumous booklet on Cellular Respiration by NORMAN U. MELDRUM recalls also the brief monograph on Respiration in Plants by W. STILES and W. LEACH two years ago. MELDRUM's monograph, 116 pages with glossary, appendix, and index, contains seven chapters: Introduction; dehydrogenase systems; the work of WARBURG; oxidases, peroxidases, and catalase; the cytochrome system; the glutathione system; and modern developments. The author purposely omits the oxidation-reduction potential problems as too controversial, inaccurate, and too difficult to interpret.

The STILES and LEACH monograph is of almost the same size, 124 pages. There is an introductory chapter, and chapters on the respiration of normal plants under aerobic conditions, anaerobic respiration, and the mechanism of respiration.

The MELDRUM monograph may be obtained from Methuen & Co. Ltd., 36 Essex St. W. C., London, at 3*s.* 6*d.*, postage extra. The other is published by the Dial Press, 152 West 13th St., New York, at $1.50 per copy. Both are useful little books which should be in the hands of plant physiologists.

Plant Biochemistry.—An Introduction to Plant Biochemistry by CATHERINE CASSELS STEELE, is published by G. Bell & Sons Ltd., London. There are 27 chapters, which fall into seven parts. The general ground covered is indicated by the titles of these larger divisions: Introduction; alcohols, fatty acids, fats and oils; aldehydes, ketones, and carbohydrates; plant acids; proteins and related compounds; cyclic compounds; and plant metabolism. The earlier sections deal with the nature of the constituents rather than with their biochemical origin. A large amount of information has been brought together, and the book will no doubt find many interested readers. The quoted price is 15 shillings, net.

Reshaping Agriculture.—This little book by Dr. O. W. WILLCOX looks forward to the time when agriculture will be so efficient that 80 per cent. of our rural population will not be needed as tillers of the soil. He believes that we now have enough technical knowledge about plants that if it were all utilized in practice there would be but one limiting factor on yield,—the "quantity of life," or innate capacity for growth belonging to the species. He goes to the opposite extreme of the old Malthusian doctrine of ultimate starvation from inadequate food supplies to a plethora of abundance that needs to be "managed," socially controlled. Chapter VI deals with the abolishing of weather hazards in farming, a subject that ought to interest the present administration in Washington. The chapter on social intervention in agriculture should be read by everyone interested in the real welfare of mankind; for it is only by knowing what "new dealers" are thinking that defense mechanisms against foolishness can be constructed. Dr. WILLCOX seems privately to feel that botanists and ordinary agriculturists have done scant courtesy to his ideas concerning agrobiology. As this little volume, Reshaping Agriculture, published by W. W. Norton & Co., New York, costs only $2.00, it would at least be easily possible to follow the author's excursion into the "era of abundance" and its "implementation." The reshaping of agriculture probably has about as good a chance to succeed as the reshaping of business, or the reshaping of human

nature. The reviewer feels that this agricultural social-economic millennium is about as far away as the millennium of the Apocalypse.

Horticultural Plant Breeding.—This volume, Grundriss der gärtnerischen Pflanzenzüchtung, is a general work on applied genetics. The author is H. KAPPERT, of the Landwirtschaftliche Hochschule, Berlin. The sixteen sections cover the range of Mendelian behavior, mutation phenomena, chimaeras, graft hybrids, selection, pure lines, inbreeding, heterosis, etc. The Mendelian behavior is considered in detail, although the entire work is condensed into small compass, 148 pages. Horticulturists will find it a useful work on breeding. It comes in stiff brochure binding at RM 6.80 per copy. The publisher is Paul Parey, Berlin.

Pathology of Mitosis.—A monograph entitled Pathologie der Mitose has been prepared by Dr. GEORG POLLITZER, of the University of Vienna. The discussion deals with the abnormalities of mitosis, mainly of animal cells, although a number of cases are described from plant materials. The author first considers the morphology of abnormal karyokinesis, then disturbances in the cell division rhythm, the comparative etiology of abnormal mitoses, actinic effects, chemical effects, and the effects of electric currents. A brief chapter at the close considers the problem of specificity, that is, specific cell reactions to disturbing factors. The work is published by Gebrüder Borntraeger, Berlin, at RM 16.20 bound in cloth.

Mammalian Red Cells.—Although not in the domain of plant physiology, attention is called to the publication of volume 6 of the Protoplasma Monographien which are published by Gebrüder Borntraeger, Berlin. The author is Dr. ERIC PONDER, New York University, and the complete title of the book is The Mammalian Red Cell and the Properties of Haemolytic Systems. The earlier chapters discuss methods of counting corpuscles, their dimensions, shape and structure, chemical composition and metabolism, permeability and the phenomenon of osmotic haemolysis. Later chapters consider the properties of haemolytic systems, inhibition and acceleration of haemolysis, resistance series, systems containing sensitizing agents, and miscellaneous forms of haemolysis. The price is RM 22.5 per copy, with cloth binding.

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